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Supporting Online Material for

Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation

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Materials and Methods

Small molecule exposure. Larval zebrafish were raised on a 14 hour/10 hour light/dark cycle at 28.5^{o}C. At four days post fertilization (dpf), a single larva was pipetted into each of 80 wells of a 96-well plate (7701-1651; Whatman, Clifton, NJ) containing 650 µL of standard embryo water (0.3 g/L Instant Ocean, 1 mg/L methylene blue, pH 7.0). Larvae were then exposed to small molecules beginning at ~96 hours post fertilization (hpf) by directly pipetting a 10 mM stock solution (in DMSO) into each well (10 animals per compound) for a final chemical concentration of ~10-30 µM and no more than 0.3% DMSO. This concentration of DMSO has no effect on behavior. Small molecules were obtained from the following libraries and screened at the following final concentrations: 1) Spectrum library from Microsource Discovery at ~30 µM (Gaylordsville, CT); 2) Prestwick library from Prestwick Chemical at ~15 µM (Illkirch, France); 3) neurotransmitter (cat#2810), ion channel (cat#2805), and orphan ligand libraries (cat#2825) from Biomol International at ~15 µM (now Enzo Life Sciences, International; Plymouth Meeting, PA); 4) Sigma LOPAC library at ~10 µM (Sigma-Aldrich, St. Louis, MO). Behavioral recording began at 11 PM, approximately 110 hpf.

Locomotor activity analysis. The behavioral assay is adapted from (*S1*). Locomotor activity was monitored for 60 hours using an automated video tracking system (Videotrack; Viewpoint Life Sciences, Montreal, Quebec, Canada) with a Dinion one-third inch Monochrome camera (model LTC0385; Bosch, Fairpoint, NY) fitted with a fixed-angle megapixel lens (M5018-MP; Computar) and infrared filter. The movement of each larva was recorded using the Videotrack quantization mode, and data from two cameras were collected in alternating minutes by one computer. The 96-well plate and camera were housed inside a custom-modified Zebrabox (Viewpoint, LifeSciences) that was continuously illuminated with infrared lights and illuminated with white lights from 9 AM to 11 PM. The 96-well plate was housed in a chamber filled with circulating water to maintain a constant temperature of 28.5^{o}C. The Videotrack threshold parameters for detection used were: detection threshold, 40; burst, 25; freeze, 4; bin size, 60 seconds.

Data analysis. The data was processed using custom PERL, Visual Basic Macros for Microsoft (Seattle, WA) Excel, and Matlab (version R2007a; The Mathworks, Inc). Any one minute bin with less than 0.1 second of total movement was defined as one minute of rest; a rest bout was defined as a continuous string of rest minutes (*S1*). Rest latency was defined as the length of time from lights on or off to the start of the first rest bout. Total activity was defined as the average amount of detected activity in seconds, including all rest bouts. Waking activity was defined as the total amount of detected activity, excluding all one minute periods of rest. These parameters were calculated for each experimental day and night, standardized to camera-matched DMSO controls, and

expressed as +/- standard deviations from controls for hierarchical clustering (see below). We calculated the inter-assay variability as a coefficient of variation (standard deviation of all DMSO controls/mean of DMSO controls*100) for each measurement. For non-normal distributions, including rest length, rest latency, and wakingactivity, the coefficient of variation was calculated following a Box-Cox transformation to a normal distribution. The coefficient of variations (expressed as a %) are as follows: total rest, day 1, 42.3%, day 2, 42.1%, night 1, 24.4%, night 2, 19.9%; rest bouts, day 1, 27.3%, day 2, 21.9%, night 1, 16.7%, night 2, 11.3%; rest bout length, day 1, 14.6%, day 2, 12.9%, night 1, 15.6%, night 2, 11.3%; rest latency, day 1, 35.7%, day 2, 45.8%, night 1, 13.2%, night 2, 20.0%; total activity, day 1, 35.5%, day 2, 28.1%, night 1, 36.3%, night 2, 29.9%; waking activity, day 1, 15.5%, day 2, 17.9%, night 1, 18.9%, night 2, 15.6%. Much of the variability is due normal inter-individual variation.

To generate time series data for average rest per 10 minutes and average waking activity per 10 minutes (red traces, e.g. Figure 2B-F), these two parameters were averaged across all 10 replicate animals in 10 minute intervals and then normalized to camera matched controls by dividing each 10 minute rest and 10 minute waking activity measure by the mean daytime rest and activity for camera matched controls, respectively. For comparison, 10 representative sets of DMSO controls (10 larvae each set; e.g. blue traces in Figure 2B-F) are plotted in each time series. The same DMSO controls are shown in all graphs for clarity.

To identify compounds that significantly altered behavior, each parameter was compared to camera-matched DMSO controls by Student's t-test. Compounds were chosen for further analysis if either: 1) the compound altered the same parameter in the same direction at a p value $< 10^{-5}$ for two consecutive days or nights, 2) the compound affected a behavioral parameter at $p<10^{-10}$ within the first 24 experimental hours, or 3) the compound altered rest latency at $p<0.001$ for two consecutive days or nights but had little effect on other parameters. These parameters were chosen to select the largest manageable set of small molecules with the strongest and most consistent effects on behavior while minimizing false positives and compounds with spurious short-term behavioral alterations. Biological targets were assigned to compounds based on annotations in the Pharmaprojects, Drugbank, Drugs@FDA, and Leadscope Marketed Drugs databases, as well as information supplied by the library vendors and detailed manual literature searches for each compound.

Hierarchical and k-means clustering analysis. Clustering analysis was performed with Cluster 3.0 (adapted by Michiel de Hoon from Cluster, written by Michael Eisen, (*S2*)) and visualized using TreeView (written by Michael Eisen, (*S2*)). Hierarchical clustering was performed using Pearson uncentered correlation and average linkage. The parameters were weighted as follows: total rest, 1.0; rest bouts, 0.8; rest bout length 0.3, rest latency, 1.0; total activity and waking activity, 1.0. K-means clustering was performed using k=24 and Euclidean distance. To determine the relative position of the k-clusters, the average vector for each cluster was calculated, and these 24 vectors were hierarchically clustered using Euclidean distance.

Correlation Analysis: All correlation analysis was performed in Matlab (version R2007a; The Mathworks, Inc). The weighted, uncentered Pearson's correlation coefficients were calculated for all compound pairs. Compounds were assigned to pharmacological classes as described above. Compounds were considered to share targets if both their target assignment and activity (i.e. agonist or antagonist) overlapped for at least one target.

Rank Correlation Analysis: To perform rank correlation analysis for adrenergic antagonists and ERG blocking drugs, the average weighted uncentered Pearson's correlation coefficient between each compound of the identified cluster and every other small molecule was calculated. Each small molecule was then assigned a rank based on their mean correlation, with rank 1 being the highest mean correlation. Compounds that showed ERG or adrenergic antagonist activity were identified by their pharmacological assignments, and the Kolmogorov-Smirnov statistic was used to assign the p value for whether ERG or adrenergic antagonist compounds were enriched among the higher ranking compounds. The rank correlations were graphically represented in Treeview (*S2*).

Dose Correlation Analysis. Compounds were ordered from Sigma-Aldrich (St. Louis, MO), dissolved in DMSO to a stock concentration of 30 or 45 mM and tested in the locomotor activity assay at final concentrations of 0.15, 0.45, 1.5, 4.5, 15, and 45 µM, or 0.1, 0.3, 1.0, 10, and 30 µM (0.3% DMSO final concentration). Each concentration was tested on 10-20 larvae. To generate the fingerprint for each concentration, the behavioral parameter measurements were standardized to camera-matched DMSO controls and expressed as +/- standard deviations from controls. Two-tailed t-test assessments (Bonferroni corrected for multiple comparisons) determined statistically significant differences of each measurement relative to camera-matched controls. The correlation matrices were generated by calculating the weighted, uncentered Pearson's correlation coefficient between the fingerprints for all concentration pairs. The fingerprint, significance, and correlation matrix heat maps were generated using Matlab (version R2007a; The Mathworks, Inc.).

MAO inhibitor assay. MAO-B activity was assayed as described (*S3*).

Supplemental Texts 1-7

Supplemental Text 1. Psychotropic Drug Discovery

The limitations of in vitro drug screening are particularly acute for the development of drugs that modulate the central nervous system (CNS). Surveys of drug discovery success show that CNS drugs take longer to develop (12.6 years versus 6.3 years for cardiovascular drugs) and fail to reach the market more often (7% success rate versus 15%) than other therapeutic indications (*S4*). The CNS poses a particular challenge to drug discovery because the brain cannot be modeled in vitro. Indeed, most modern psychotropic drugs were first developed for non-psychotropic uses and serendipitously discovered to have behavior-altering side effects (e.g. the MAO inhibitor, iproniazid, was developed to treat tuberculosis and found to alter mood, and the tricyclic antidepressant imipramine was first used as an anti-histamine, (*S5*)). Furthermore, CNS drugs tend to produce undesirable side effects, including dizziness, tremors, lethargy, and seizures, which are difficult to predict at early stages of the drug discovery pipeline. Also, the mechanism of action for many CNS drugs, including bipolar medications, antidepressants, and antipsychotics, is poorly understood and in many cases may depend on a compound's complex poly-pharmacological actions across multiple neurotransmitter systems (*S6-7*). Indeed, efforts to increase efficacy and reduce side effects through the development of newer generations of drugs with more selective target profiles has to date achieved only limited success (*S8-9*).

Alternatives or complements to in vitro target-based screens include phenotype-based, whole organism screens. Such screens have several advantages. First, whole-organism screens need not be limited to single, well-validated targets. In fact, whole organism screens can identify efficacious compounds with complex mechanisms of action. Second, unwanted side effects, an obstacle in any drug discovery pipeline, can be detected at earlier stages, even during the primary screening process. Third, compounds that produce a behavioral effect are bio-available, another major stumbling block to in vitro based drug discovery pipelines. In principle, whole organism phenotypic screening could replace serendipity, but the requirement for large numbers of phenotyped animals is usually cost and time prohibitive. Behavioral screening in zebrafish larvae offers a cost-effective model system for psychotropic drug characterization and discovery.

Supplemental Text 2. Dose

Choosing the compound concentration is a challenge for any large-scale small molecule screen. Effective doses can vary among compounds, but it is impractical in most cases to screen at multiple concentrations. The decision to perform our screen in the 10-30 µM range was based on the following considerations. First, we were guided by several previous studies. Developmental screens in zebrafish used concentrations of 10-50 µM (*S10-12*), single compound behavioral studies in zebrafish were done at 1-100 µM (*S13-19*), and the connectivity map cell-based screen was performed at 10 µM (*S20, 21*). Second, we performed a pilot screen of 96 compounds and found that only 10% of

the compounds were overtly toxic at the 30 μM range, a percentage deemed acceptable for screening. Third, dose response curves on selected compounds indicated that a concentration of 10-30 μM was an effective range with consistent behavioral effects for most compounds (Table S1 and Fig. S4). Specifically, we found that fingerprints for the same compound were strongly correlated (25/29 cases) across the statistically effective concentration range (Fig. S4). These results show that the behavioral fingerprints are broadly stable across multiple doses.

Supplemental Text 3. Therapeutic Classes of Drugs Induce Correlated Behaviors

To test the potential of the clustering analysis, we determined whether compounds that share targets are more likely to produce similar phenotypes (Fig. S5A-B). Expanding this correlation analysis to 50 categories of drugs, we found, with few exceptions, that the correlations among related classes of compounds were much larger than chance (Fig. S5C). In many cases, every intra-class pair-wise correlation was larger than the zero target correlation (see Figure Legend S5 for definitions of category abbreviations; + indicates agonist, - indicates antagonist), including the ADR-A2+ (median correlation = 0.853; range = 0.352-0.974), SNRI (median = 0.775; range = 0.453-0.956), 5HT-1A+ (median = 0.791; range = 0.463-0.958), NEURO- (median = 0.759; range = 0.587-0.894), DOPA-D1+ (median = 0.885; range = 0.766-0.927), and CHA-NA+ (median = 0.833; range = 0.519-0.985) classes (Fig S5C). In other examples, intra-class pair-wise correlations were much larger except for a few outliers, including the SSRI (median = 0.767 ; range = 0.262-0.963), 5HT-1A- (median = 0.769; range = -0.02-0.930), 5HT-2- (median = 0.698; range = 0.187-0.977), DOPA-D2+ (median = 0.727; range = 0.072-0.982), GABA- (median = 0.722; range = -0.05-0.972), HIST-H1-(ERG) (median = 0.722; range = 0.229-0.911), and NMDA- (median = 0.651; range = -0.419-0.971) classes (Fig. S5C). Not all classes had higher correlations than the no target class, including the adrenergic reuptake class (ADR-U; median = 0.257; range = -0.50-0.959) and the sodium channel antagonist class (CHA-NA-; median = -0.031; range = -0.513 – 0.913). This may reflect the heterogeneity of secondary targets for the compounds of these classes.

Supplemental Text 4. Polypharmacology.

Many compounds have multiple, potentially non-overlapping targets. In such cases, one would expect that not all compounds that share targets produce similar phenotypes (Fig. S5A-C). For example, outliers of the correlation analysis might reflect the difficulty in assigning compounds to single classes when in fact they have multiple targets. This observation can be used to identify potential polypharmacological effects (Figs. S5C and S7). For example, NMDA antagonist (NMDA-) outliers included compounds that also bind to σ-opioid receptors (e.g. 3-methoxymorphinan), the GABA antagonist (GABA-) outlier tert-butyl-bicyclophosphorothionate (TBPS) also potently blocks chloride channels, the serotonin receptor 1A antagonist (5HT-1A-) outlier S(-)UH-301 is also a dopamine D2/3 receptor agonist, and the dopamine D2 receptor agonist (DOPA-D2+) outlier pergolide is also a serotonin receptor agonist (Fig S5C). Our correlation analysis can also uncover higher order associations among drugs with multiple targets, such as the high correlation between drugs that share dopamine reuptake inhibition and acetylcholine antagonism (Figure S7A-B). Furthermore, the clustering analysis can

reveal unexpected associations between annotated drug classes. For example, a cluster of known α1-adrenergic receptor antagonists (ifenprodil, terazosin, and 5-methyl-urapidil) also cluster with several serotonin receptor modulators, including NAN-190, RU-24969, WAY-100635, spirotraxine, and buspirone (Fig. S7C-D). Intriguingly, buspirone and spirotraxine do not strongly bind to α1-adrenergic receptors in vitro yet can show in vivo adrenergic activity in specific pharmacological contexts (*22*). These results show that behavioral profiling can uncover associations between multi-target drugs whose properties are not fully predicted by in vitro studies.

Supplemental Text 5. Pharmacological Conservation (Figs. S8-15).
We systematically compared the behavioral effects of agonists and antagonists of the major neurotransmitter systems between zebrafish and mammals. In most cases, confidence in the conservation between zebrafish and mammals is enhanced when multiple compounds known to affect the same target elicit similar phenotypes and when agonist/antagonist pairs elicit opposing phenotypes. We discuss each class in more detail below.

Adrenaline (Fig. S8). In zebrafish, α2-adrenergic agonists decreased wakefulness with little effect on rest, while activation of β-adrenergic receptors selectively reduced total rest at night. Conversely, β-adrenergic receptor antagonists increased total rest. α1-adrenergic antagonists gave a "mixed" phenotype of increased waking activity but also increased rest. These effects are broadly consistent with those observed in mammals (*S23-35*).

Serotonin (Fig. S9). The analysis of serotonin-regulating drugs is complicated because most, if not all, serotonin-modulating drugs have considerable affinity for non-serotonin receptors, especially dopaminergic and adrenergic receptors. Thus, the drugs chosen for the table in Figure S9 combine serotonin-signaling selectivity, consistency of phenotype, and reproducibility (i.e. multiple hits within the screen of the same or similar compounds). As often as possible, the drugs shown have known mammalian phenotypes (*S36-55*). The complexity of the phenotypes we observed with serotonergic agonists and antagonists is similar to the controversial and contradictory nature of the mammalian literature pertaining to these drugs. This may reflect the difficulty in isolating the drugs' effects on serotonin signaling from other secondary effects, differences between short-term and long-term exposure, or complex signaling interactions among different populations of serotonergic neurons in the brain. For example, in mammals agonists and antagonists of serotonin receptor 1A both cause short-term reduction of sleep and increase in wakefulness followed by longer-term increases in sleep and reduced activity (*S36-40, S45-47*). In long-term experiments, both classes of drugs increased rest in zebrafish. Only buspirone had an observed short-term increase in waking during the first night of the experiment (black arrow in Fig. S9). Serotonin receptor 2/3 antagonists have paradoxical effects in mammals, increasing both exploratory behavior and sleep (*S38, S44, S49-52*). Similarly, both waking activity and total rest were increased in zebrafish. Finally, SSRIs consistently decreased waking activity and increased rest in zebrafish; in human patients, SSRIs

often improve insomnia in depressed patients but can have biphasic effects on sleep, with long-term decreases in sleep in some mammalian models (*S53-55*).

Dopamine (Fig. S10). With one notable exception, dopamine receptor agonists and antagonists produce similar phenotypes in mammals and zebrafish (*S56-67*). For example, the D2-receptor agonists consistently reduced waking activity and increased rest in zebrafish, as they do at low concentrations in mammals (*S60-63*). This effect was receptor subtype selective, as the D2/D3 receptor agonists quinelorane and quinpirole only reduced waking activity (*S64, S65*). As another example of conservation, D2-receptor antagonists, several of which are used as anti-psychotics, increase waking activity and rest in zebrafish and mammals (*S57, S62, S67*). Previous work indicated that antipsychotics produce locomotor defects in zebrafish larvae (*S15-16*). The only clear examples of non-conserved pharmacology in our study were the dopamine D1 agonists (Fig. S10, marked in gray), all of which had little effect on waking activity but greatly increased total rest in zebrafish. In all mammalian species tested, dopamine D1 agonists increased waking (*S56-59*). This could indicate a distinct role for the zebrafish orthologs of D1 receptors in the control of behavior or that these dopamine D1 agonist drugs have altered target selectivity in zebrafish.

GABA (Fig. S11). While many of the GABA-regulating benzodiazepine drugs were toxic at the concentrations used in our screen, the GABA-A agonists GBLD-345 and avermectin B1 increased total rest and had only a small effect on waking activity (*S68*). Although only represented by a single hit in the screen, the GABA-B agonist CGP-13501 (Fig. S11, marked in gray) slightly increased waking activity at night with very modest increases in rest during the day, even though this drug is hypnotic in mammals (*S69*). In contrast, many GABA-A antagonists dramatically increased waking activity, possibly reflecting pro-convulsant effects of these drugs on zebrafish larvae, which is consistent with the mammalian literature (*S70-72*) and with previous work in zebrafish (*S13, S17, S19*). Tracazolate, an anxiolytic acting as an allosteric modulator of GABA-A receptors, modestly increased waking activity in zebrafish; in mammals, tracazolate can both increase or decrease locomotor activity (*S73, S74*). Intriguingly, the neuroactive steroid GABA agonists allopregnanolone, pregnanolone, and alfadolone increased both total rest and waking activity specifically at night (*S75-77*). As neuroactive steroids have additional functions in mammalian brains, including modulation of NMDA receptors (*S75*), this mixed phenotype may reflect the polypharmacology of these compounds.

Melatonin (Fig. S12). Although only a few examples were present in our screen, melatonin-modulating drugs gave results that are consistent with those observed in mammals (*S78-82*) and previous work in zebrafish (*S14*). Melatonin slightly increased rest in our long-term experiments. In addition, the melatonin agonist 8-methoxy-1-propionamidotetralin decreased waking activity and increased rest. An exception was the agonist IIK7 (*S78*), which slightly increased waking activity at night and had small effects on rest.

Histamine (Fig. S13). Histamine antagonists identified in our screen tended to increase rest, decrease waking activity, or both. This increased rest in zebrafish is consistent with the mammalian literature, although it should be noted that anti-histamine drugs that do not cross the blood-brain barrier in humans, such as loratadine, tend to be less sedating than the older generation of drugs that do cross the blood-brain barrier (*S83-87*). Our work is also consistent with previous work on anti-histamines in zebrafish larvae (*S13, S18*). Notably, the anti-histamine diphenylpyraline increased waking activity, consistent with reports of psychostimulatory effects in rodents (*S87*). Also, as noted in the main text and Figures 4C-D and S18, anti-histamines that block the human ether-a-go-go related potassium channel (ERG) consistently increased waking activity at night, with modest effects on rest in zebrafish larvae.

Adenosine (Fig. S14). Consistent with mammals, adenosine receptor agonists increased rest in zebrafish larvae, while adenosine receptor A1 antagonists increased waking activity and reduced rest (*S88-93*). Although behavioral effects of the adenosine A3 antagonist MRS-1220 are unknown in mammals, it decreased waking activity and increased rest in zebrafish. This phenotype is consistent with its inhibitory effects on MAO (Figure 3D).

Glutamate (Fig. S15). All NMDA receptor antagonists identified in the screen dramatically increased waking activity during the day and night, consistent with the effects observed in mammals (*S94, S95*). Muscarinic glutamate receptor 5 (mGluR5) antagonists selectively increased rest, with little effect on waking activity. Antagonists of mGluR5 can block drug-induced hyperlocomotion, down-regulate bursting of prefrontal cortical neurons in awake rats, and have anxiolytic properties in rodents (*S96, S97*).

Supplemental Text 6. Non-overlapping Regulation of Rest/Wake States.

Many pharmacological agents selectively altered waking activity, rest latency, or total rest. For example, verapamil related L-type calcium channel inhibitors increased rest with no effect on waking activity (Fig. S16), while β-adrenergic agonists, including clenbuterol and fenoterol, decreased rest only (Fig. S8). Non-competitive NMDA receptor antagonists, including the psychotomimetics dizocilpine (MK-801) and L-701324, predominantly increased waking activity with minimal effects on total rest (Figs. 2C and S15), the D2/D3 agonist, ropinirole, predominantly decreased waking activity only (Fig. S10), and podocarpatrien-3-one analogs predominantly altered only rest latency (Fig. 4A). These single parameter effects can also be selective for day versus night. For example, many anti-inflammatory compounds only increased waking activity during the day (Figs. 2B, 4B and S17), while ERG-blocking drugs increased waking activity at night (Figs. 2E, 4C-D, S18). Finally, some compounds can affect waking activity and total rest in opposite directions (e.g. increase both waking activity and total rest). For example, the α1-adrenergic antagonists ifenprodil, the antimalarial quinacrine, and the steroidal GABA agonist alfadolone, increased both waking activity and total rest (Figs S7C, S11). These observations suggest that the behavioral parameters of waking activity, total rest, and rest latency during the day and the night can be under the control of distinct pharmacological mechanisms.

Supplemental Text 7. High Throughput Behavioral Screening in Practice

Behavioral profiling in zebrafish is highly efficient. Our current zebrafish behavior room (~200 sq. ft.) has 16 cameras that can each observe 80 wells of a 96-well plate, for a total of 1280 larvae. Using our original screening method of 10 larvae per compound and including 10 control wells for each camera, we can screen 112 compounds (16 cameras x 7 drugs per camera) every three days, or 224 compounds per week. Using the latest videotracking software, which can track all 96 wells of the plate, and cutting the number of larvae per drug to eight expands our capacity to 352 compounds per week (16 cameras X 11 drugs per camera X 2 runs per week). A single technician can set up the entire behavioral room in less than 4 hours, including fish husbandry, larval pipetting, drug dispensing, and software setup (an 8 hour commitment per week). Expanding the screen to 115 cameras and 5 technicians (a total commitment of 40 hours per week) would increase the throughput to more than 10,000 compounds per month and more than 100,000 per year.

Supplemental Figure and Table Legends

Figure S1. Expanded hierarchical clustering analysis. This is an expanded version of Figure 2A in which the name of each chemical in the clustergram can be seen. Each row represents a different chemical, and each column represents a behavioral measurement. From left to right, these measurements are: the rest total, the number of rest bouts, the rest bout length, the rest latency, the total activity, and the waking activity. The black bars indicate night measurements; the white bars indicate day measurements. Yellow indicates that the value is increased relative to controls; blue indicates that the value is decreased, as in Figure 2A. The measurements are normalized as standard deviations from control values.

Figure S2. Hierarchical and k-means clustering yield similar cluster architectures. Each row represents a different chemical, and each column represents a behavioral measurement. From left to right, these measurements are: the rest total, the number of rest bouts, the rest bout length, the rest latency, the total activity, and the waking activity. The black bars indicate night measurements; the white bars indicate day measurements. Yellow indicates that the value is increased relative to controls; blue indicates that the value is decreased, as in Figure 2A. The measurements are normalized as standard deviations from control values.

Figure S3. Expanded k-means clustergram. This is an expanded version of Figure S2 in which the name of each chemical in the clustergram can be seen. Each row represents a different chemical, and each column represents a behavioral measurement. From left to right, these measurements are: the rest total, the number of rest bouts, the rest bout length, the rest latency, the total activity, and the waking activity. The black bars indicate night measurements; the white bars indicate day measurements. Yellow indicates that the value is increased relative to controls; blue indicates that the value is decreased, as in Figure 2A. The measurements are normalized as standard deviations from control values.

Figure S4. Behavioral fingerprints are stable across a range of doses. Select compounds were tested for behavioral effects at multiple concentrations (see Table S1 for additional examples and summary data). First, the fingerprints for each dose were calculated as standard deviations from DMSO controls (‘Fingerprint’, left panels). In general, these fingerprints are stable across multiple concentrations until the dose is too low to elicit a phenotype. To assess the stability of single drug fingerprints over multiple concentrations, the Pearson uncentered correlation coefficient was calculated pairwise between each dose (‘correlation matrix’, right panels). With the exception of pergolide and several other ergoline derivatives, which have distinct fingerprints at high versus low concentrations, these examples have relatively stable fingerprints from the highest dose tested (45 μM) to the lowest effective doses (150 nM for NAN-190 and

betamethasone, 450 nM for ropinirole, 1.5 μM for clonidine and buspirone), as indicated by the high correlations (red boxes). See Materials and Methods for additional information.

Figure S5. Compounds that share biological targets have highly correlated behavioral fingerprints. (A) The distribution of all pair-wise correlation coefficients (Pearson weighted, uncentered; see Materials and Methods) for compounds that share at least 1 target (blue) is shifted to higher values compared to compounds that share 0 targets (magenta). This skewing is strong despite the fact that these compounds can have many divergent targets in addition to the shared target. Each pair of compounds is represented once, even if they share multiple targets. (B) Box-whisker plot comparing correlations between compounds with 0 shared targets, compounds with at least 1 shared target, and repeats of the same compound from different chemical libraries. For each measure, the blue box represents the interquartile range between the 25^{th} and 75^{th} percentiles; the red line marks the sample median; the whiskers extend from the box ends to values within 1.5 times the interquartile distance; red crosses mark outlier values beyond the 1.5 interquartile distance from the box ends. (C) Box-whisker plots of correlation coefficients within biologically related classes of compounds. For comparison, the mean correlation of compounds that share 0 targets is plotted as a green line (correlation = 0.266). The number of compounds used for comparisons within each class is indicated (N). See supplemental text 3 for a detailed discussion of specific classes. For all classes, a (-) sign indicates an inhibitor/antagonist and a (+) sign indicates an agonist. The classes are: α-adrenergic-receptor 1, ADR-A1; α-adrenergic receptor-2, ADR-A2; β-adrenergic, ADR-B; noradrenaline reuptake inhibitor, ADR-U-; serotonin/noradrenaline reuptake inhibitor, SNRI; selective serotonin reuptake inhibitor, SSRI; serotonin receptor-1A, 5HT-1A; serotonin receptor-1D, 5HT-1D; serotonin receptor-2, 5HT-2; serotonin receptor-2/3, 5HT-2/3; serotonin receptor 4, 5HT-4; monoamine oxidase inhibitor, MAO-; neurotransmitter inhibitor (reserpine class), NEURO-; dopamine receptor, DOPA; dopamine D1 receptor, DOPA-D1; dopamine D2 receptor, DOPA-D2; dopamine D2/3 receptor, DOPA-D2/3; dopamine D3 receptor, DOPA-D3; dopamine D4 receptor, DOPA-D4; γ-butyric acid receptor, GABA; histamine H1 receptor, HIST-H1; ether-a-go-go-related potassium channel, ERG; adenosine signaling, ADS; metabotropic glutamate receptor 5, mGLUR5; N-methyl-D-aspartic acid receptor, NMDA; acetylcholinesterase inhibitor, ACH-ES- IRev, irreversible; Rev, reversible; muscarinic acetylcholine receptor; ACH-M; nicotinic acetylcholine receptor, ACH-N; acetylcholine synthesis, ACH-SY; L-type calcium channel; CHA-CA-L; sodium channel, CHA-NA; peroxisome proliferator-activated receptor, alpha, PPAR-A.

Figure S6. Examples of compounds that share biological targets and/or structural similarity that give similar behavioral profiles. (A) Structurally divergent selective serotonin reuptake inhibitors (SSRIs) similarly decrease waking activity and increase rest. (B) Structurally related insecticides dramatically increase waking activity independent of the time of day. The graphs represent the normalized waking activity and total rest for behavior-altering compounds (red trace; average of 10 larvae) and representative controls (10 blue traces; average of 10 larvae each).

Figure S7. Cluster analysis of multi-target compounds. A dopamine reuptake inhibitor (DOPA-U) with muscarinic acetylcholine antagonist (ACH-M-) activity does not correlate well with (A) compounds with only DOPA-U activity or (B) compounds with only ACH-M- activity. It does, however, show high correlation with other compounds known to share both properties (B). (C) α1-adrenergic receptor antagonists (marked in blue) form a large cluster with serotonin modulators (marked in green) that are known to also have α1- adrenergic receptor antagonist activity in vivo. (D) Rank-correlations of compounds across the entire dataset compared to the α1-adrenergic receptor antagonist cluster of ifenprodil, terazosin, and 5-methyl-urapidil enriches for compounds with α1-adrenergic receptor antagonism. In the rank-graph, the black lines indicate compounds with known α1-adrenergic antagonism; red indicates high correlation, green indicates low correlation. Compounds with α1-adrenergic antagonism are highly enriched in the top ranks ($p < 10^{-23}$; see Materials and Methods).

Figure S8-15. Comparison of the effects of neuroactive drugs on rest/wake states in zebrafish to the effects in mammals. The graphs represent the waking activity and rest for representative agonists and antagonists of each class (red trace; average of 10 larvae) and DMSO controls (10 blue traces; average of 10 larvae each). Each plotted example is typical for the entire class of compounds. The tables provide a more comprehensive list of agonists and antagonists of each receptor subtype. The tables summarize the overall direction and magnitude of the observed effects of the drugs on zebrafish wake and rest behavior (up arrow, increased; down arrow, decreased; a dashed line indicates no change; a small arrow indicates a smaller relative effect, while a large arrow indicates a larger relative effect). See supplemental text 5 for a more detailed discussion of each phenotype.

Figure S16. The L-type calcium channel inhibitor, YS-035, dose dependently increases rest with minimal effects on waking activity. (A) Waking activity and rest graphs are shown for three structurally related L-type calcium channel inhibitors (red trace = average of 10 larvae) and representative DMSO controls (10 blue traces = average of 10 larvae). (B) YS-035 increases the time spent at rest during both the day (red) and night (blue) in a dose dependent fashion. Each point indicates the average minutes of rest per hour for 10 larvae at each YS-035 concentration. The error bars represent +/- SEM. (C) YS-035 has little or no effect on waking activity at all concentrations tested, suggesting that muscle function is not dramatically perturbed. Each point indicates the average seconds of waking activity every 10 minutes during the day (red) or night (blue) for 10 larvae at each YS-035 concentration. Error bars represent +/- SEM (B, C).

Figure S17. Examples of immunomodulators that co-cluster as daytime-waking activity enhancers. (A) The table lists anti-inflammatory agents that selectively increased daytime waking activity (expanded examples from Figure 4B). (B) Waking activity and rest graphs are shown for compounds from each major class (red trace = average of 10 larvae) and representative controls (10 blue traces = average of 10 larvae).

Figure S18. ERG inhibitors, but not non-ERG blocking anti-histamine analogs, dose dependently increase waking activity at night. (A) Waking activity and rest graphs are

shown for two ERG-blocking compounds (red trace = average of 10 larvae) and representative controls (10 blue traces = average of 10 larvae). Both compounds increased waking activity at night without affecting rest. Psora-4, which blocks Kv1.3 shaker channels, increased nighttime waking activity and also strongly decreased rest, a phenotype distinct from ERG blockers. Thus, the ERG phenotype is not the result of general potassium channel misregulation. (B) Dose response curves for ERG blocking and ERG non-blocking analogs are shown. Each point indicates the waking activity at night (seconds per minute) averaged for 10 larvae. Cisapride and dofetilide, which have ERG blocking activity, dose-dependently increased nighttime wakefulness, while the non-ERG blocking histamine H1 receptor antagonists fexofenadine and cetirizine had no effect on wakefulness. Another non-ERG blocking anti-histamine, loratadine, reduced waking activity. Error bars represent +/- SEM. The 0 M concentration is a DMSO control.

Table S1. Summary of dose response experiments for selected compounds indicates fingerprints are stable across several concentrations. This table is an expansion of the dose response fingerprints shown in Fig. S4. Note that only ergoline-derived compounds (bottom of table) have complex dose dependent effects (i.e. different nontoxic fingerprints at high versus low concentrations). This complex dose dependence is likely due to the high affinity that many ergoline drugs have for multiple receptor families and sub-types, including dopamine, serotonin, and adrenergic receptors (for example, see (*S98*)).

Figure S1

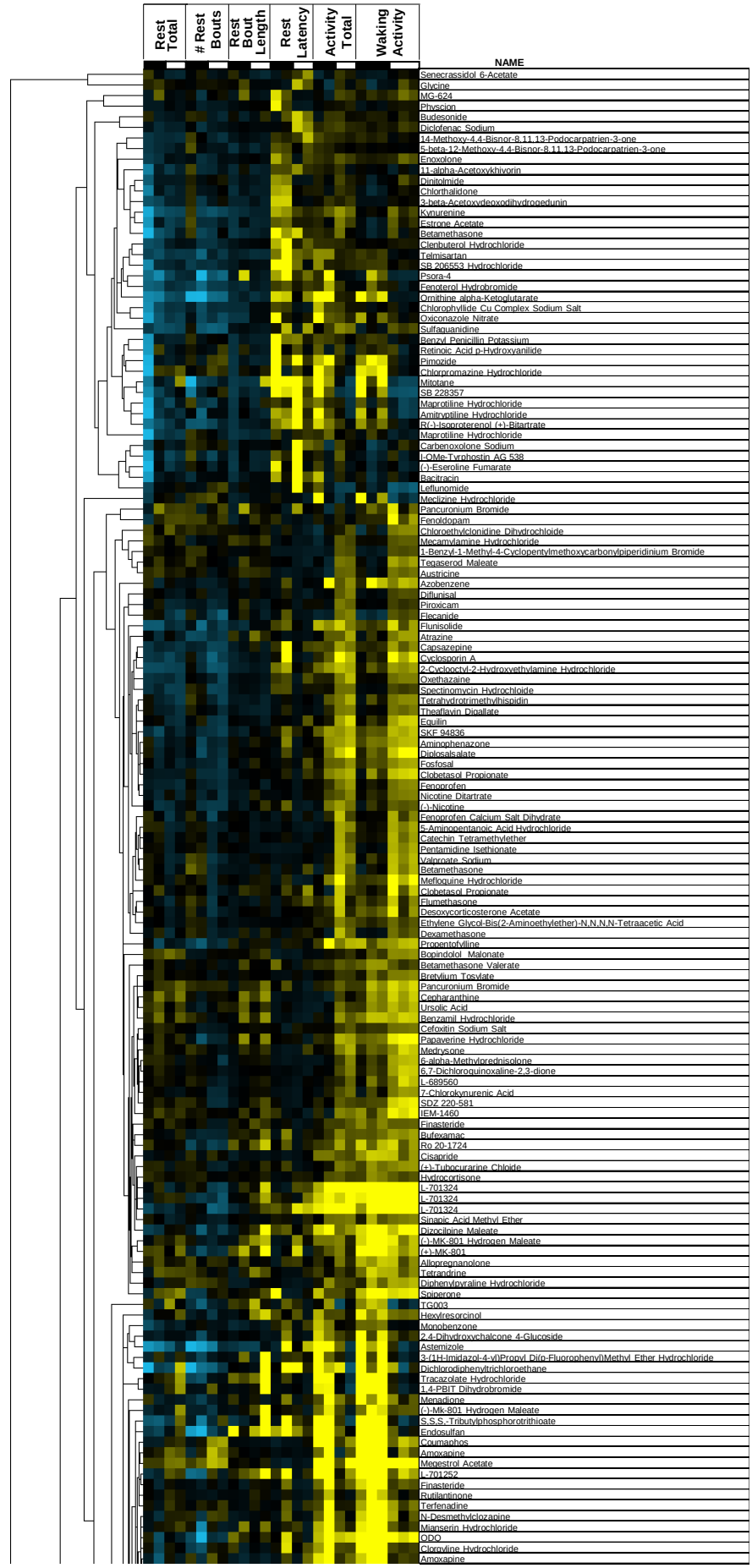
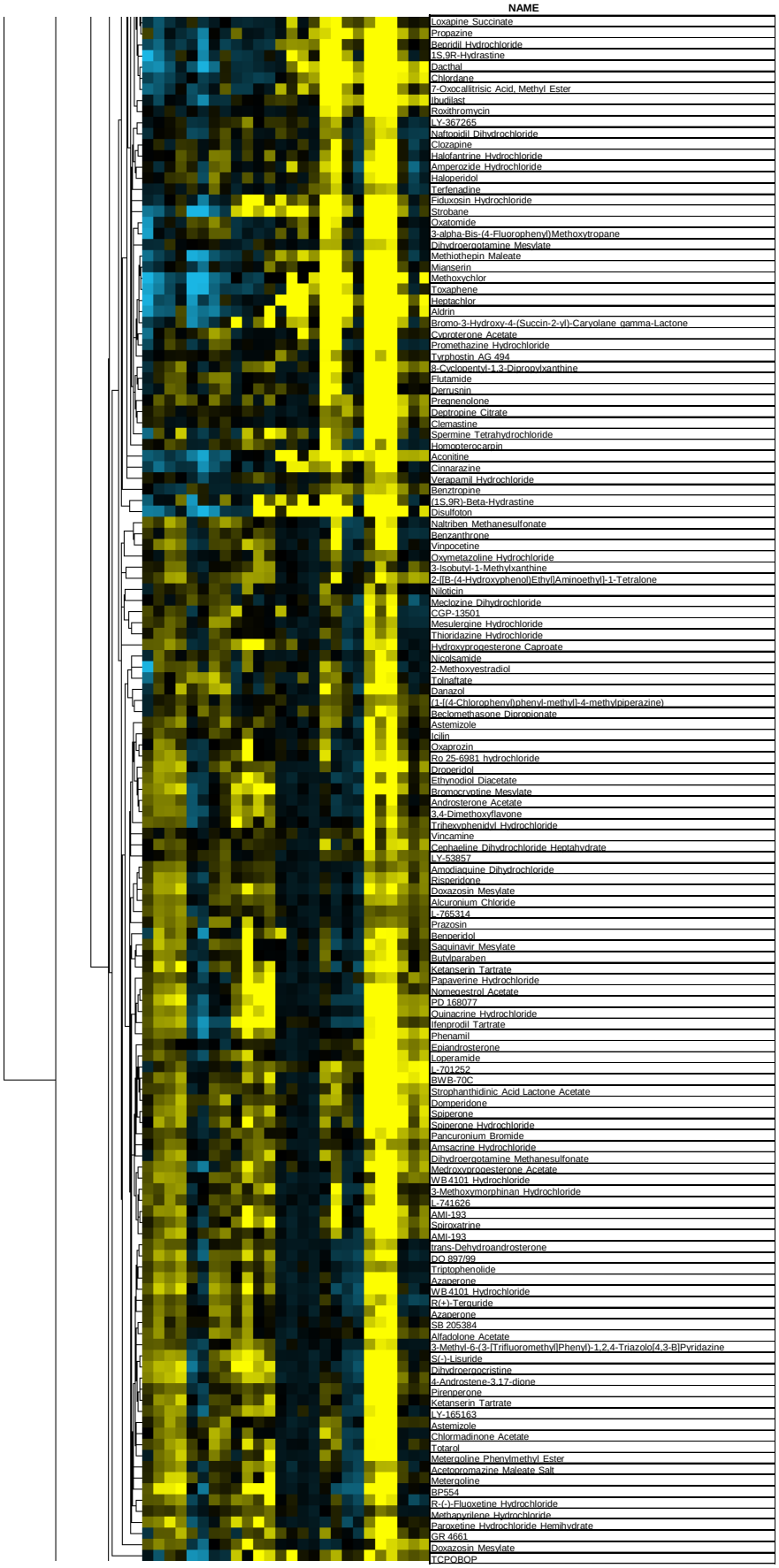

Rest Total
Rest Bouts
Rest Bout Length
Rest Latency
Activity Total
Waking Activity
NAME
Senecrassidol 6-Acetate
Glycine
MG-624
Physcion
Budesonide
Diclofenac Sodium
14-Methoxy-4,4-Bisnor-8,11,13-Podocarpatrien-3-one
5-beta-12-Methoxy-4,4-Bisnor-8,11,13-Podocarpatrien-3-one
Enoxolone
11-alpha-Acetoxykhivorin
Dinitolmide
Chlorthalidone
3-beta-Acetoxydeoxodihydrogedunin
Kynurenine
Estrone Acetate
Betamethasone
Clenbuterol Hydrochloride
Telmisartan
SB 206553 Hydrochloride
Psora-4
Fenoterol Hydrobromide
Ornithine alpha-Ketoglutarate
Chlorophyllide Cu Complex Sodium Salt
Oxiconazole Nitrate
Sulfaguanidine
Benzyl Penicillin Potassium
Retinoic Acid p-Hydroxyanilide
Pimozide
Chlorpromazine Hydrochloride
Mitotane
SB 228357
Maprotiline Hydrochloride
Amitryptiline Hydrochloride
R(-)-Isoproterenol (+)-Bitartrate
Maprotiline Hydrochloride
Carbenoxolone Sodium
I-OMe-Tyrphostin AG 538
(-)-Eseroline Fumarate
Bacitracin
Leflunomide
Meclizine Hydrochloride
Pancuronium Bromide
Fenoldopam
Chloroethylclonidine Dihydrochloide
Mecamylamine Hydrochloride
1-Benzyl-1-Methyl-4-Cyclopentylmethoxycarbonylpiperidinium Bromide
Tegaserod Maleate
Austricine
Azobenzene
Diflunisal
Piroxicam
Flecanide
Flunisolide
Atrazine
Capsazepine
Cyclosporin A
2-Cyclooctyl-2-Hydroxyethylamine Hydrochloride
Oxethazaine
Spectinomycin Hydrochloide
Tetrahydrotrimethylhispidin
Theaflavin Digallate
Equilin
SKF 94836
Aminophenazone
Diplosalsalate
Fosfosal
Clobetasol Propionate
Fenoprofen
Nicotine Ditartrate
(-)-Nicotine
Fenoprofen Calcium Salt Dihydrate
5-Aminopentanoic Acid Hydrochloride
Catechin Tetramethylether
Pentamidine Isethionate
Valproate Sodium
Betamethasone
Mefloquine Hydrochloride
Clobetasol Propionate
Flumethasone
Desoxycorticosterone Acetate
Ethylene Glycol-Bis(2-Aminoethylether)-N,N,N,N-Tetraacetic Acid
Dexamethasone
Propentofylline
Bopindolol Malonate
Betamethasone Valerate
Bretylium Tosylate
Pancuronium Bromide
Cepharanthine
Ursolic Acid
Benzamil Hydrochloride
Cefoxitin Sodium Salt
Papaverine Hydrochloride
Medrysone
6-alpha-Methylprednisolone
6,7-Dichloroquinoxaline-2,3-dione
L-689560
7-Chlorokynurenic Acid
SDZ 220-581
IEM-1460
Finasteride
Bufexamac
Ro 20-1724
Cisapride
(+)-Tubocurarine Chloide
Hydrocortisone
L-701324
L-701324
L-701324
Sinapic Acid Methyl Ether
Dizocilpine Maleate
(-)-MK-801 Hydrogen Maleate
(+)-MK-801
Allopregnanolone
Tetrandrine
Diphenylpyraline Hydrochloride
Spiperone
TG003
Hexylresorcinol
Monobenzone
2,4-Dihydroxychalcone 4-Glucoside
Astemizole
3-(1H-Imidazol-4-yl)Propyl Di(p-Fluorophenyl)Methyl Ether Hydrochloride
Dichlorodiphenyltrichloroethane
Tracazolate Hydrochloride
1,4-PBIT Dihydrobromide
Menadione
(-)-Mk-801 Hydrogen Maleate
S,S,S,-Tributylphosphorotrithioate
Endosulfan
Coumaphos
Amoxapine
Megestrol Acetate
L-701252
Finasteride
Rutilantinone
Terfenadine
N-Desmethylclozapine
Mianserin Hydrochloride
QDO
Clorgyline Hydrochloride
Amoxapine

Figure S1(Cont)



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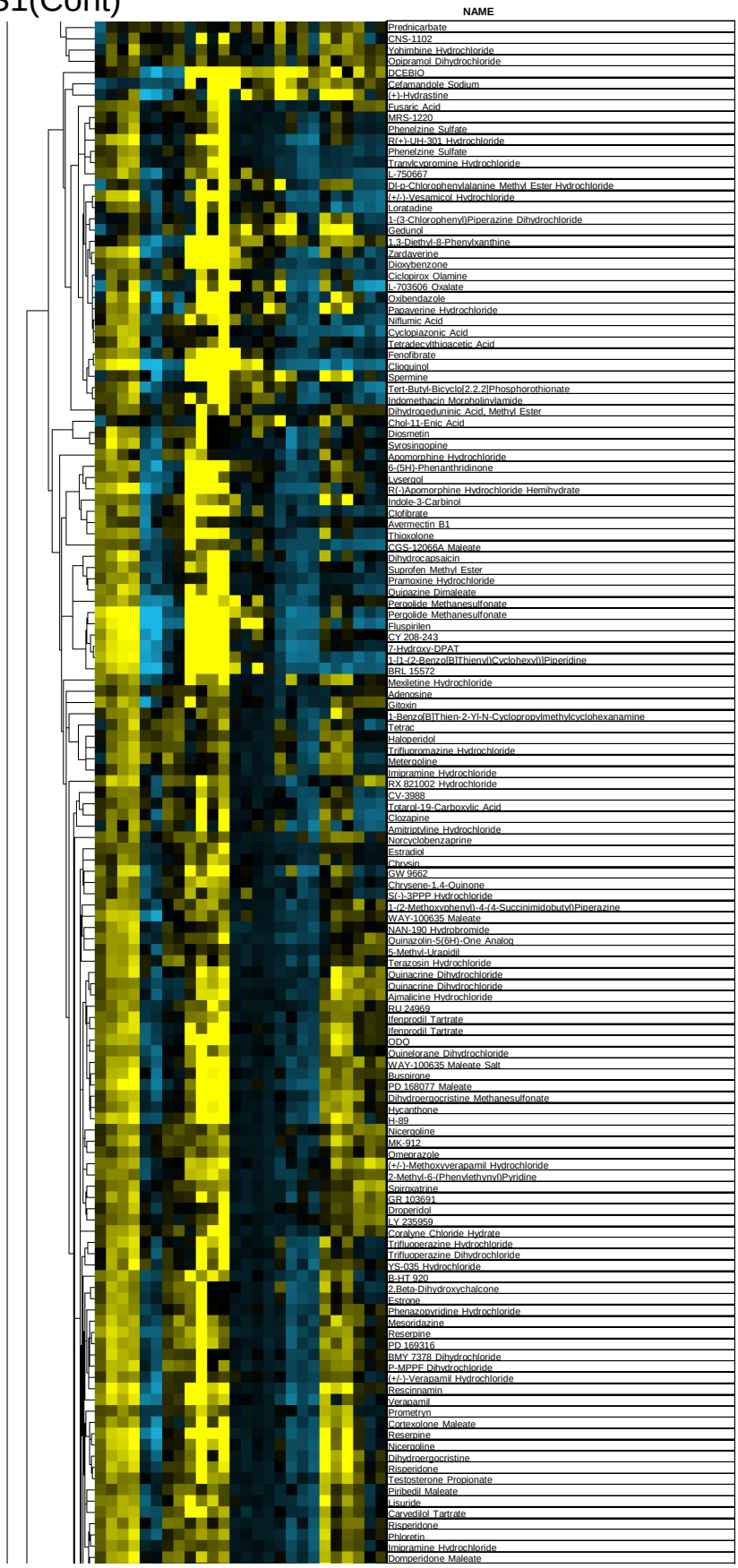

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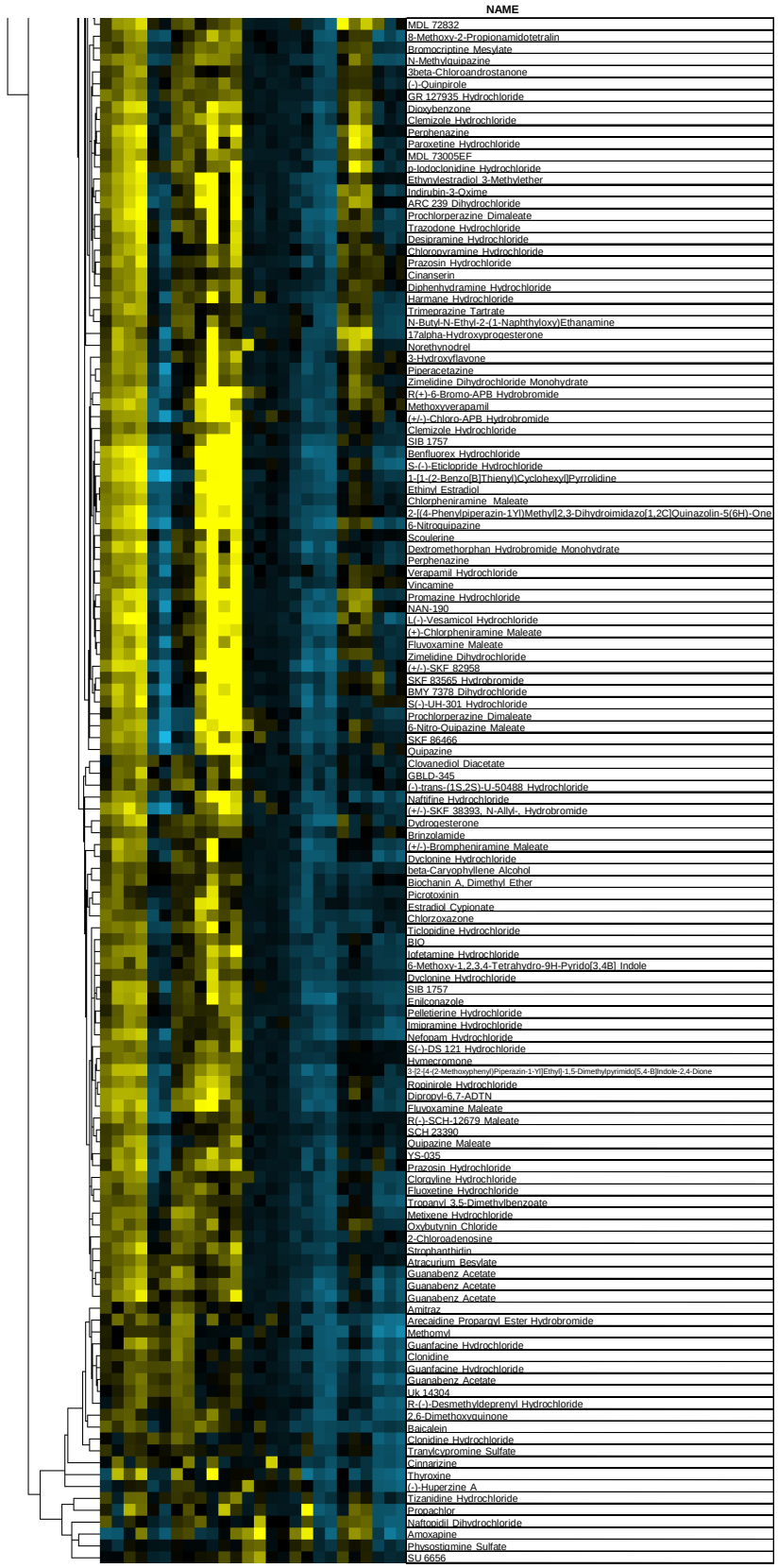

Figure S2

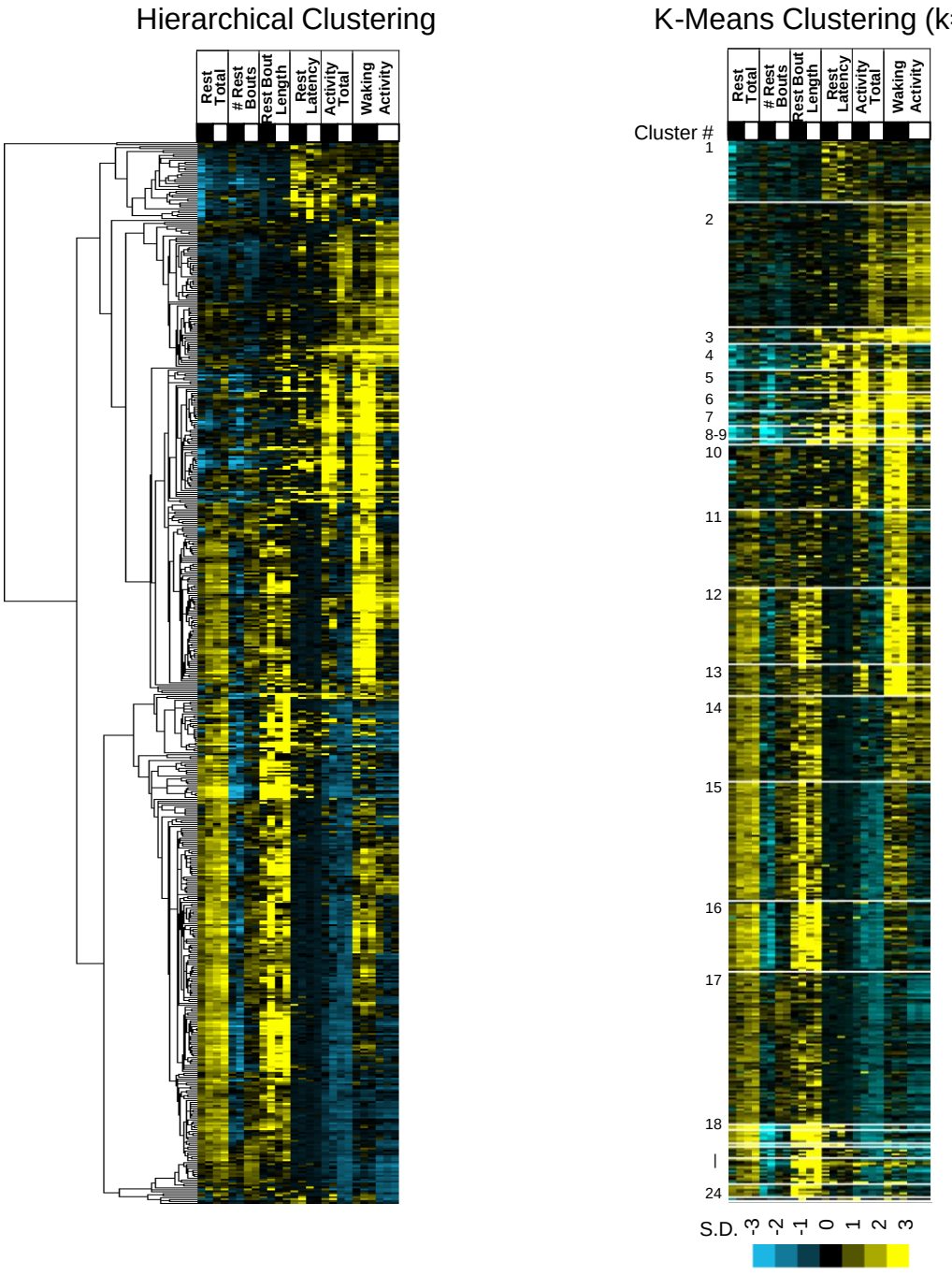
Hierarchical Clustering
K-Means Clustering (k=24)
Rest Total
Rest Bouts
Rest Bout Length
Rest Latency
Activity Total
Waking Activity
Cluster #
1
2
3
4
5
6
7
8-9
10
11
12
13
14
15
16
17
18
|
24
S.D. -3 -2 -1 0 1 2 3

Figure S3

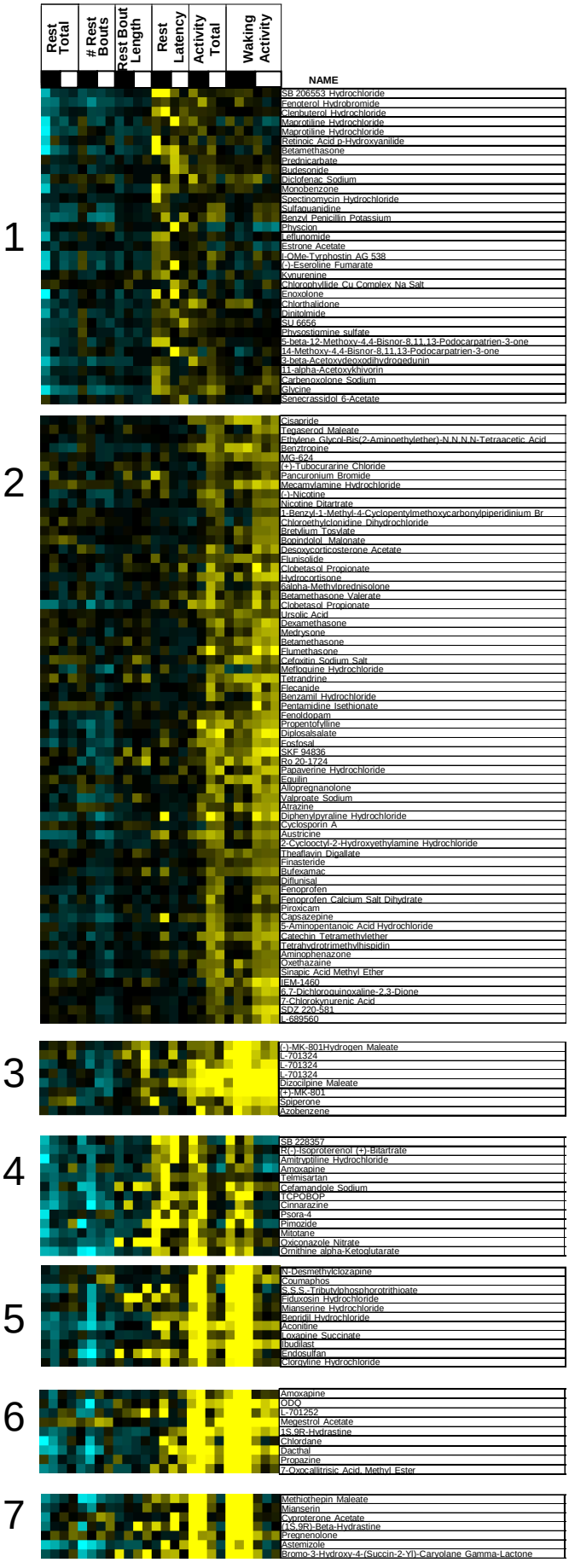
Rest Total
Rest Bouts
Rest Bout Length
Rest Latency
Activity Total
Waking Activity
NAME
1
SB 206553 Hydrochloride
Fenoterol Hydrobromide
Clenbuterol Hydrochloride
Maprotiline Hydrochloride
Maprotiline Hydrochloride
Retinoic Acid p-Hydroxyanilide
Betamethasone
Prednicarbate
Budesonide
Diclofenac Sodium
Monobenzone
Spectinomycin Hydrochloride
Sulfaguanidine
Benzyl Penicillin Potassium
Physcion
Leflunomide
Estrone Acetate
I-OMe-Tyrphostin AG 538
(-)-Eseroline Fumarate
Kynurenine
Chlorophyllide Cu Complex Na Salt
Enoxolone
Chlorthalidone
Dinitolmide
SU 6656
Physostigmine sulfate
5-beta-12-Methoxy-4,4-Bisnor-8,11,13-Podocarpatrien-3-one
14-Methoxy-4,4-Bisnor-8,11,13-Podocarpatrien-3-one
3-beta-Acetoxydeoxodihydrogedunin
11-alpha-Acetoxykhivorin
Carbenoxolone Sodium
Glycine
Senecrassidol 6-Acetate
2
Cisapride
Tegaserod Maleate
Ethylene Glycol-Bis(2-Aminoethylether)-N,N,N,N-Tetraacetic Acid
Benztropine
MG-624
(+)-Tubocurarine Chloride
Pancuronium Bromide
Mecamylamine Hydrochloride
(-)-Nicotine
Nicotine Ditartrate
1-Benzyl-1-Methyl-4-Cyclopentylmethoxycarbonylpiperidinium Br
Chloroethylclonidine Dihydrochloride
Bretylium Tosylate
Bopindolol Malonate
Desoxycorticosterone Acetate
Flunisolide
Clobetasol Propionate
Hydrocortisone
Ralpha-Methylprednisolone
Betamethasone Valerate
Clobetasol Propionate
Ursolic Acid
Dexamethasone
Medrysone
Betamethasone
Flumethasone
Cefoxitin Sodium Salt
Mefloquine Hydrochloride
Tetrandrine
Flecanide
Benzamil Hydrochloride
Pentamidine Isethionate
Fenoldopam
Propentofylline
Diplosalsalate
Fosfosal
SKF 94836
Ro 20-1724
Papaverine Hydrochloride
Equilin
Alloprednanolone
Valproate Sodium
Atrazine
Diphenylpyraline Hydrochloride
Cyclosporin A
Austricine
2-Cyclooctyl-2-Hydroxyethylamine Hydrochloride
Theaflavin Digallate
Finasteride
Bufexamac
Diflunisal
Fenoprofen
Fenoprofen Calcium Salt Dihydrate
Piroxicam
Capsazepine
5-Aminopentanoic Acid Hydrochloride
Catechin Tetramethylether
Tetrahydrotrimethylhispidin
Aminophenazone
Oxethazaine
Sinapic Acid Methyl Ether
IEM-1460
6,7-Dichloroquinoxaline-2,3-Dione
7-Chlorokynurenic Acid
SDZ 220-581
L-689560
3
(-)-MK-801Hydrogen Maleate
L-701324
L-701324
L-701324
Dizocilpine Maleate
(+)-MK-801
Spiperone
Azobenzene
4
SB 228357
R(-)-Isoproterenol (+)-Bitartrate
Amitryptiline Hydrochloride
Amoxapine
Telmisartan
Cefamandole Sodium
TCPOBOP
Cinnarazine
Psora-4
Pimozide
Mitotane
Oxiconazole Nitrate
Ornithine alpha-Ketoglutarate
5
N-Desmethylclozapine
Coumaphos
S,S,S,-Tributylphosphorotrithioate
Fiduxosin Hydrochloride
Mianserine Hydrochloride
Repridil Hydrochloride
Aconitine
Loxapine Succinate
Ibudilast
Endosulfan
Clorgyline Hydrochloride
6
Amoxapine
ODQ
L-701252
Megestrol Acetate
1S,9R-Hydrastine
Chlordane
Dacthal
Propazine
7-Oxocallitrisic Acid, Methyl Ester
7
Methiothepin Maleate
Mianserin
Cyproterone Acetate
(1S,9R)-Beta-Hydrastine
Pregnenolone
Astemizole
Bromo-3-Hydroxy-4-(Succin-2-Yl)-Caryolane Gamma-Lactone

Figure S3(Cont)

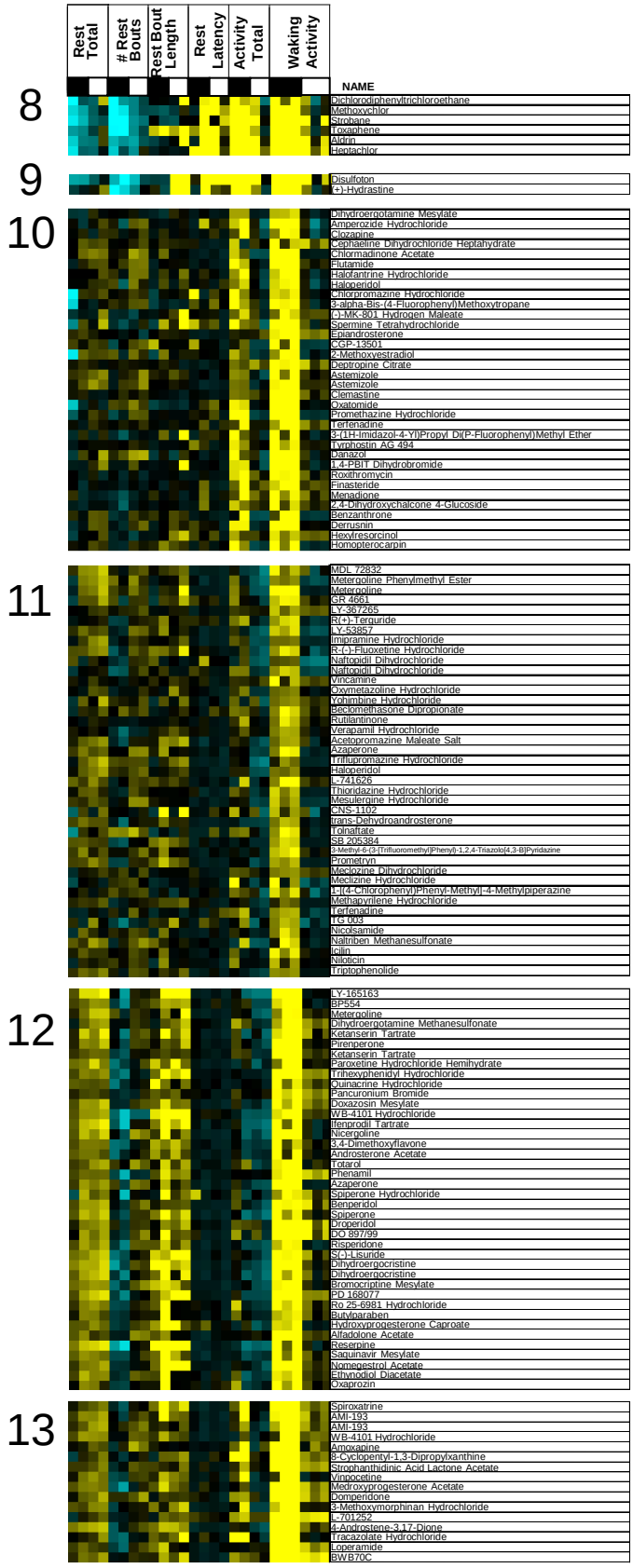

Figure S3(Cont)

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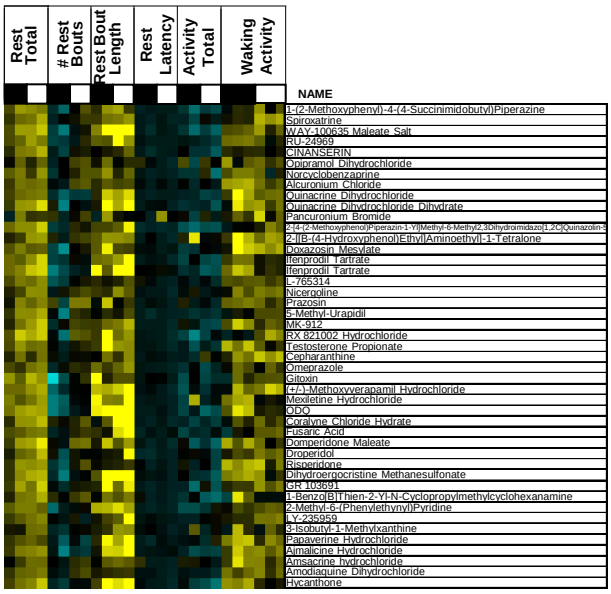

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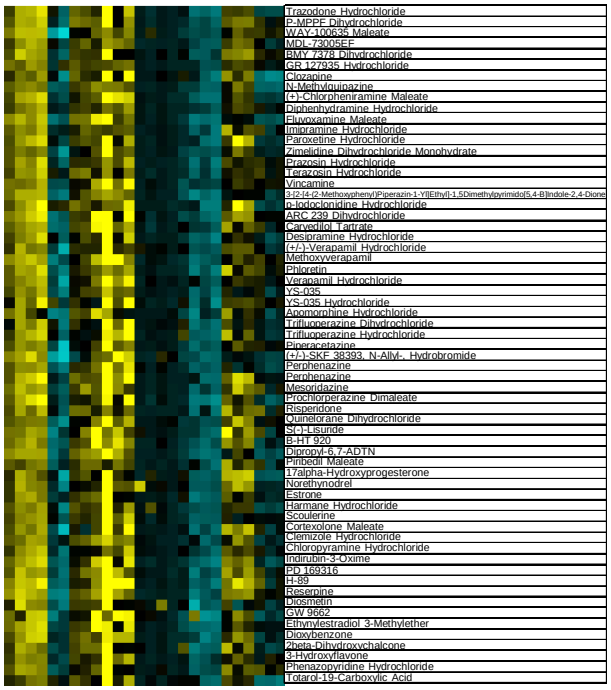

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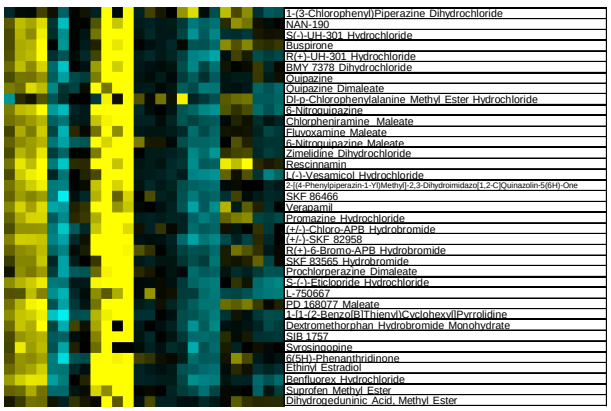

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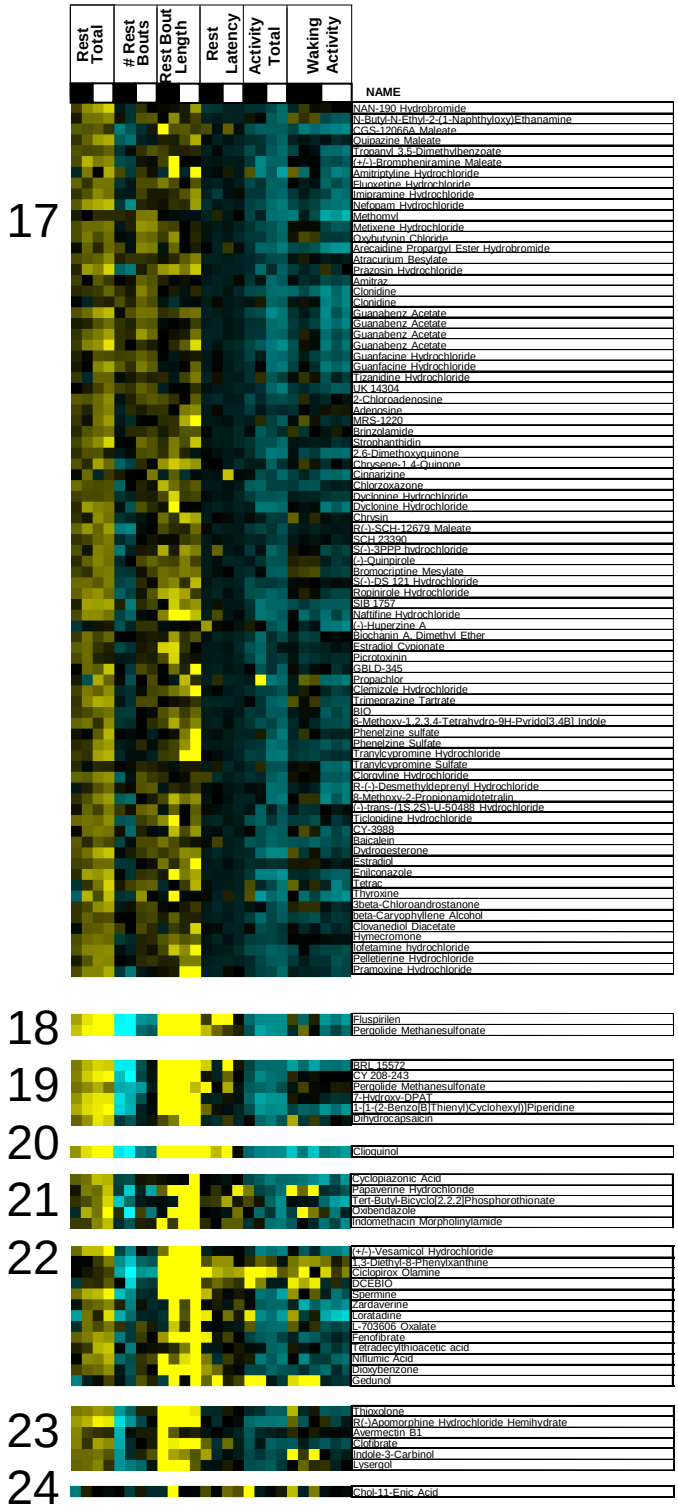


Figure S4

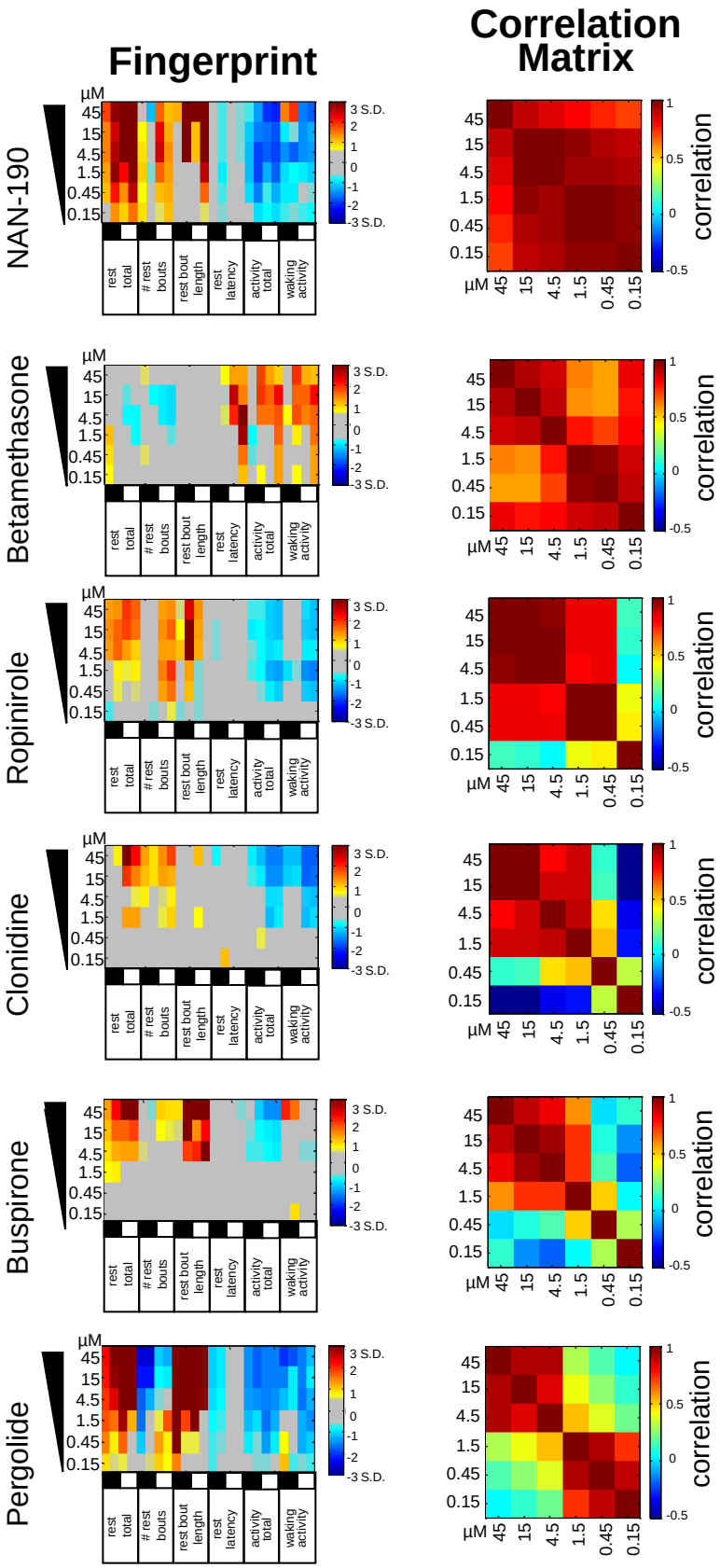

Fingerprint
Correlation Matrix
NAN-190
Betamethasone
Ropinirole
Clonidine
Buspirone
Pergolide
μM
45
15
4.5
1.5
0.45
0.15
3 S.D.
2
1
0
-1
-2
-3 S.D.
rest total
rest bouts
rest bout length
rest latency
activity total
waking activity
correlation
1
0.5
0
-0.5

Figure S5

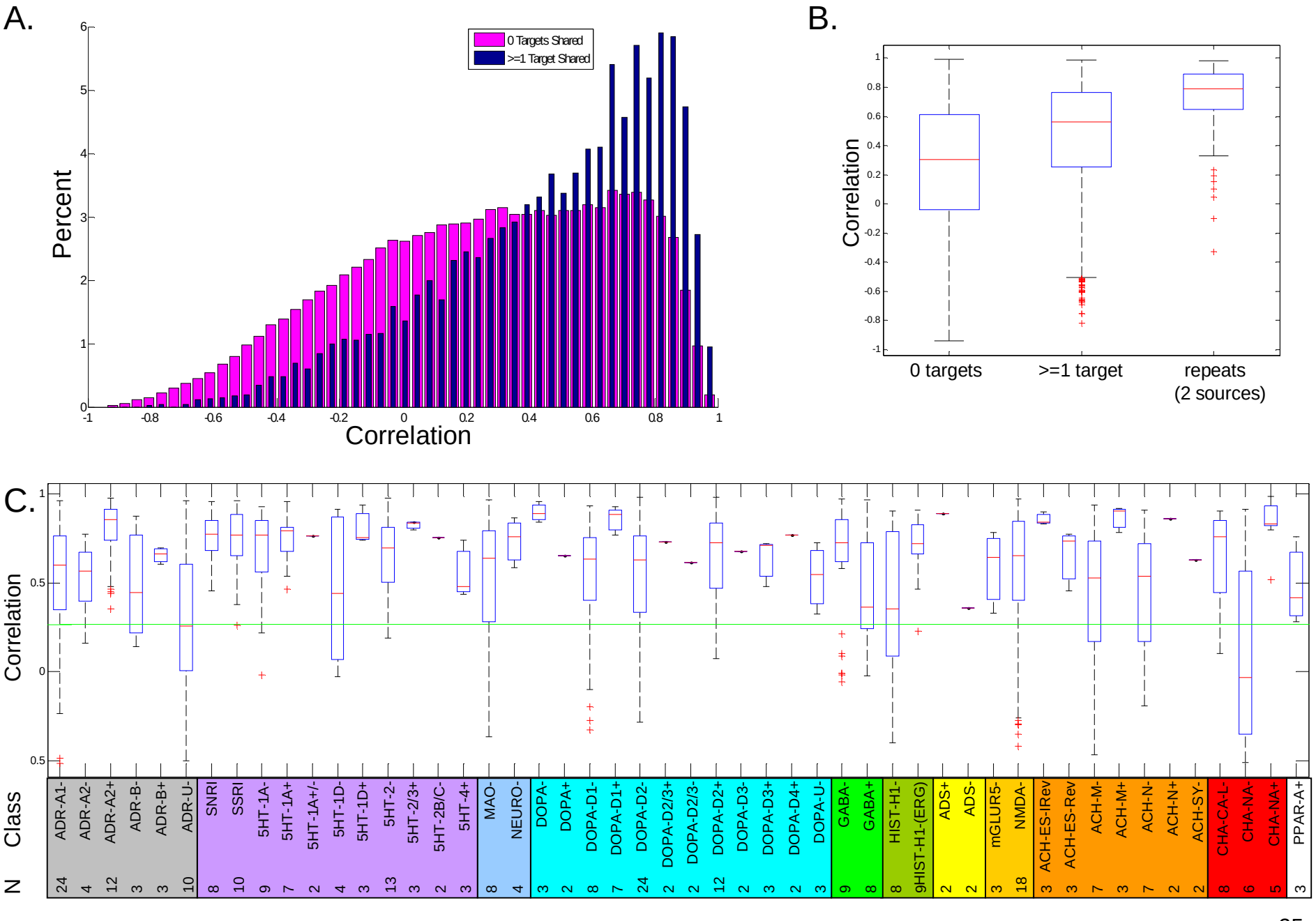
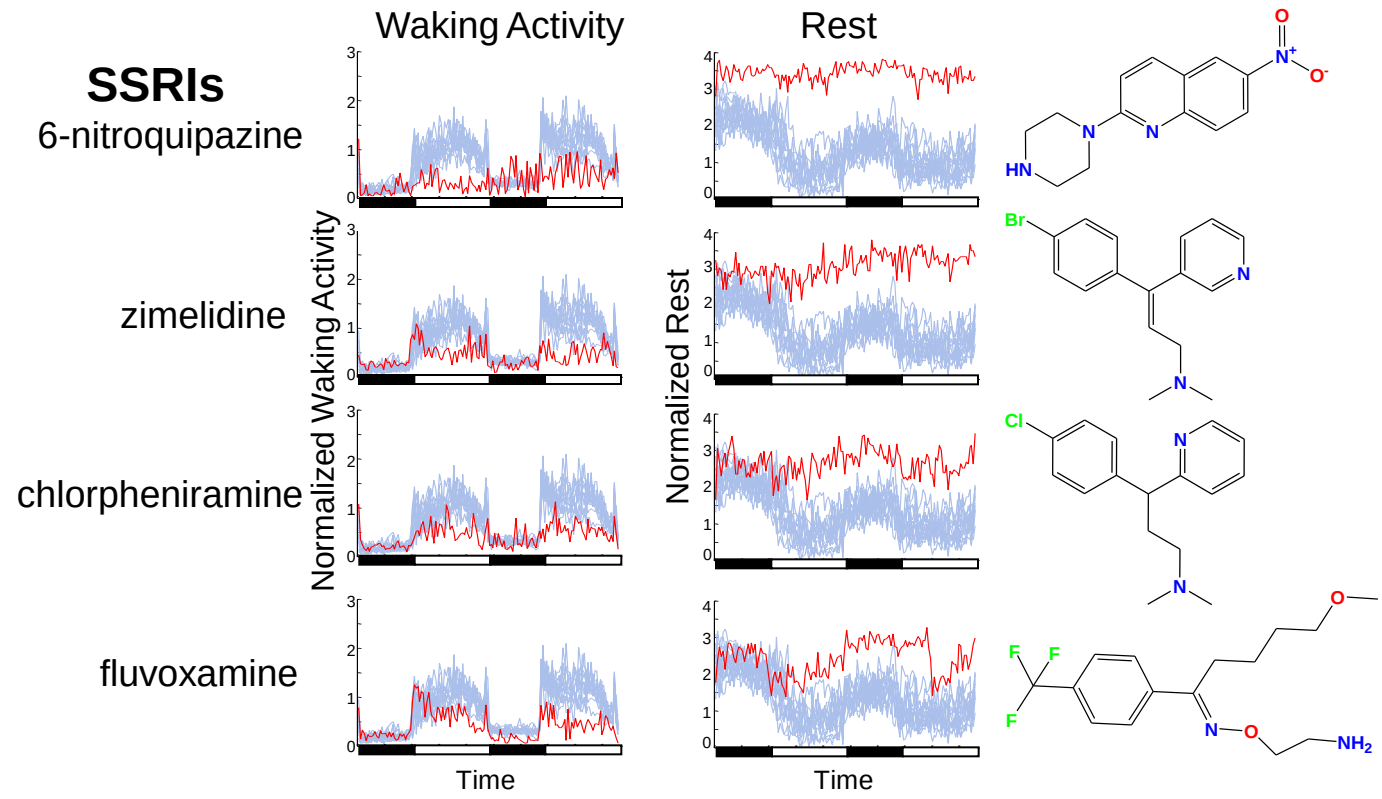

A.
0 Targets Shared
>=1 Target Shared
Percent
Correlation
B.
Correlation
0 targets
>=1 target
repeats
(2 sources)
C.
Correlation
N
Class
24 ADR-A1-
4 ADR-A2-
12 ADR-A2+
3 ADR-B-
3 ADR-B+
10 ADR-U-
8 SNRI
10 SSRI
9 5HT-1A-
7 5HT-1A+
2 5HT-1A+/-
4 5HT-1D-
3 5HT-1D+
13 5HT-2-
3 5HT-2/3+
2 5HT-2B/C-
3 5HT-4+
8 MAO-
4 NEURO-
3 DOPA-
2 DOPA+
8 DOPA-D1-
7 DOPA-D1+
24 DOPA-D2-
2 DOPA-D2/3+
2 DOPA-D2/3-
12 DOPA-D2+
2 DOPA-D3-
3 DOPA-D3+
2 DOPA-D4+
3 DOPA-U-
9 GABA-
8 GABA+
8 HIST-H1-
9HIST-H1-(ERG)
2 ADS+
2 ADS-
3 mGLUR5-
18 NMDA-
3 ACH-ES-IRev
3 ACH-ES-Rev
7 ACH-M-
3 ACH-M+
7 ACH-N-
2 ACH-N+
2 ACH-SY-
8 CHA-CA-L-
6 CHA-NA-
5 CHA-NA+
3 PPAR-A+

Figure S6

A.


Waking Activity
Rest
SSRIs
6-nitroquipazine
zimelidine
chlorpheniramine
fluvoxamine
Normalized Waking Activity
Normalized Rest
Time
Time

B.

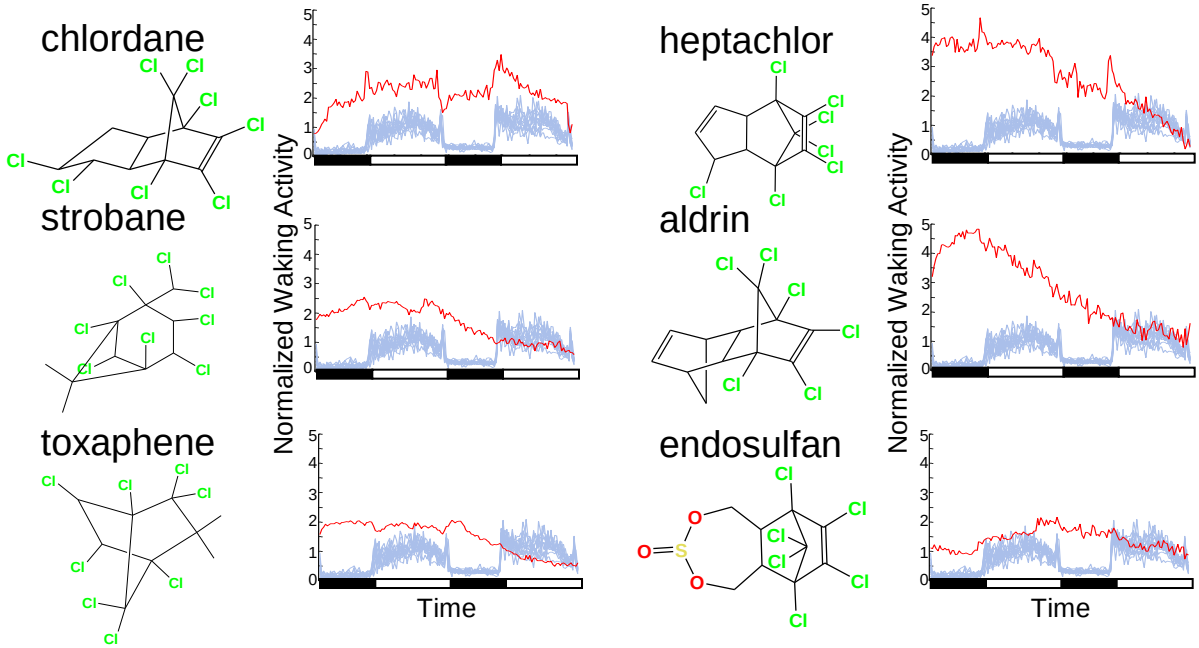
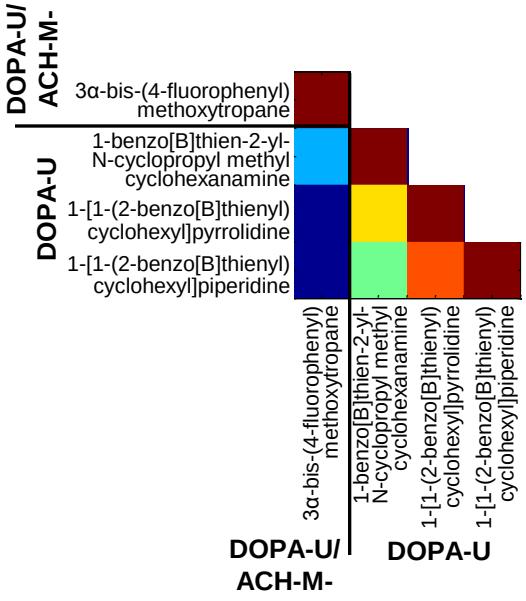
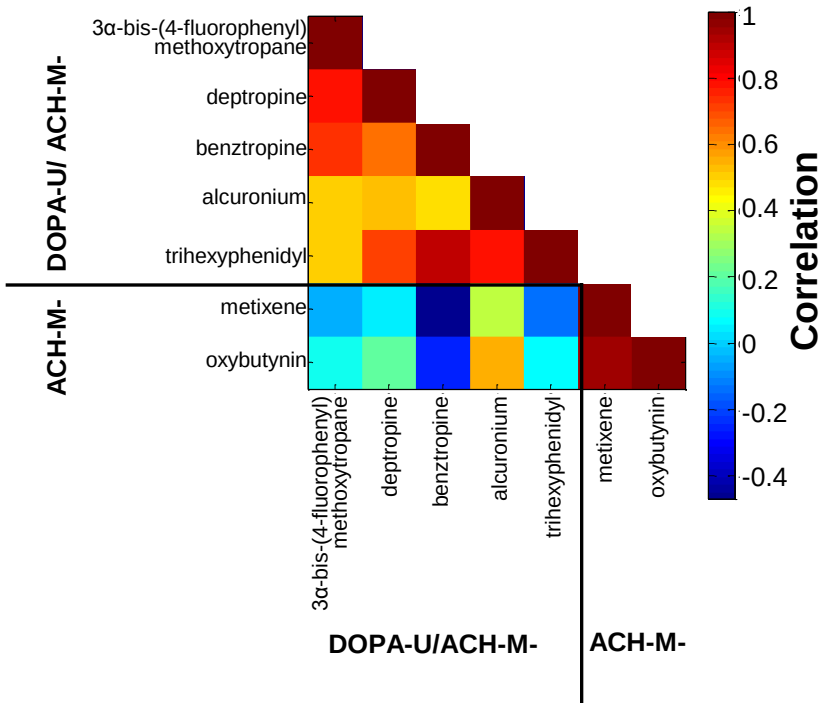

chlordane
strobane
toxaphene
heptachlor
aldrin
endosulfan
Normalized Waking Activity
Normalized Waking Activity
Time
Time

Figure S7

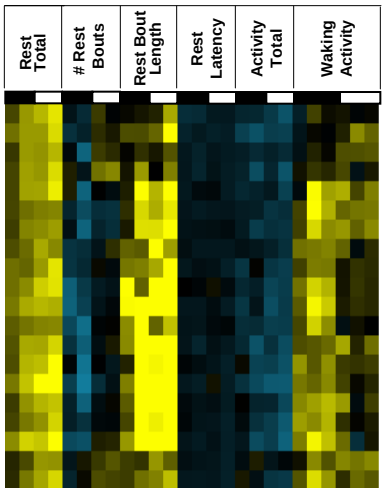
A.



B.



C.



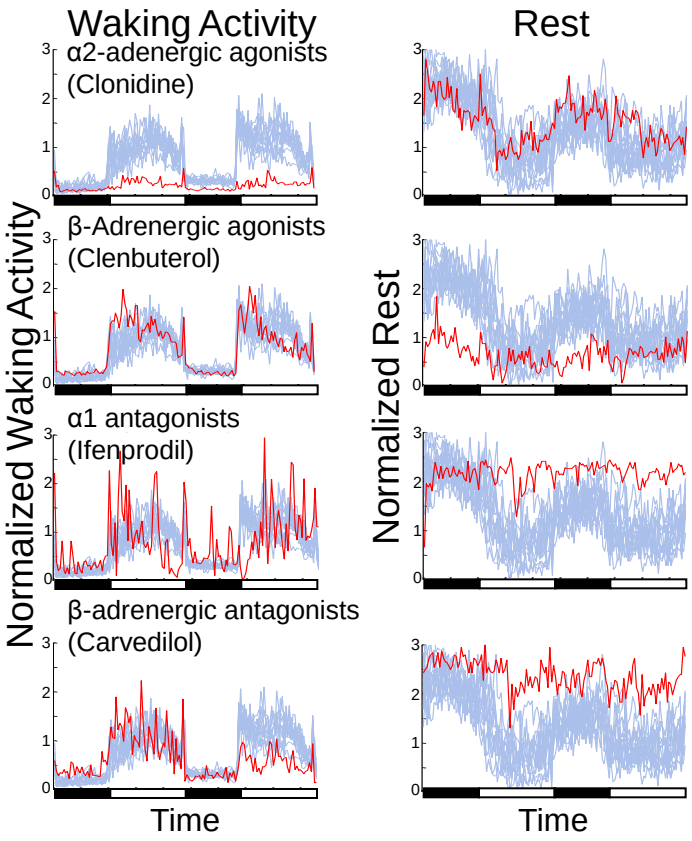
Name	Target/Indication
NAN-190	5-HT1A antagonist
Quinazolin-5(6h)-one	alpha-1 antagonist
5-methyl-urapidil	alpha-1 antagonist
Terazosin	alpha-1 antagonist
Quinacrine	antimalarial
Quinacrine	antimalarial
Ajmalicine	not annotated
RU-24969	5-HT1A agonist
Ifenprodil	alpha-1 antagonist
Ifenprodil	alpha-1 antagonist
ODQ	guanylyl cyclase
Quinelorane	dopamine agonist
WAY-100635	5-HT1A antagonist
Buspirone	5-HT1A agonist
PD 168077	dopamine agonist
Dihydroergocristine	alpha-1 antagonist
Hycanthone	not annotated
H-89	PKA antagonist
Nicergoline	alpha1-antagonist
MK-912	alpha1-antagonist

D.

Rank	Name	Mean Correlation
1	Quinacrine	0.851
2	Ifenprodil	0.848
3	WAY-100635	0.848
4	Droperidol	0.835
6	L-765314	0.831
7	Ajmalicine	0.828
8	MK-912	0.826
10	Buspirone	0.822
13	5-methyl-urapidil	0.818
15	Ifenprodil	0.817
16	Vincamine	0.817
17	H-89	0.813
18	WAY-100635	0.813
20	ARC 239	0.811
21	P-MPPF	0.810
23	Dihydroergocristine	0.809
26	NAN-190	0.802
27	RU-24969	0.802
30	NAN-190	0.797
32	Prazosin	0.793
33	M77	0.793
36	Quinazolin-5(6h)-one	0.789
37	Perphenazine	0.789
38	Nicergoline	0.785
42	BMY-7378	0.781
43	Quinacrine	0.781
45	Amodiaquine	0.778
47	Domperidone	0.770
49	Terazosin	0.769
54	Spiroxatrine	0.755
55	Risperidone	0.750

Figure S8

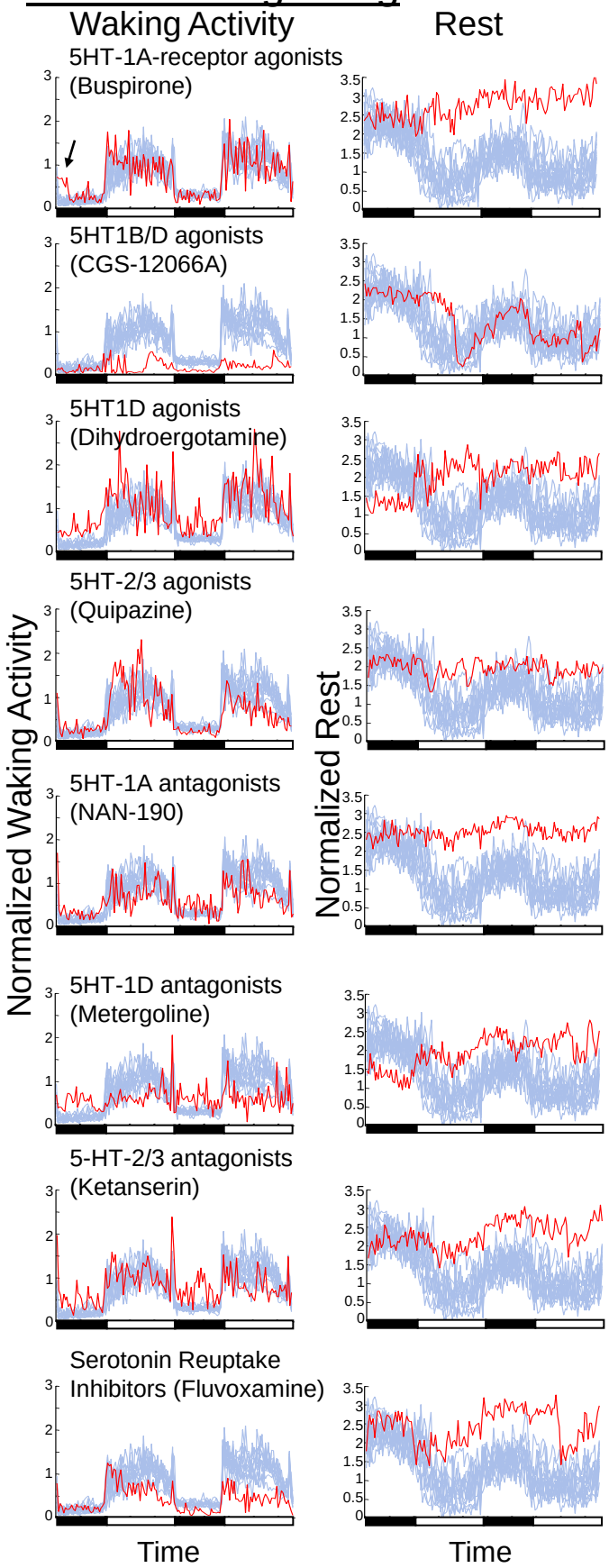
Adrenergic Signaling



Agonists	Wake	Rest	Effects in mammals	Selected References
Alpha2				
Clonidine Guanabenz Guanfacine Tizanidine UK 14304	↓	-	sedating, analgesia	(S23-S27)
Beta				
R(-)-Isoproterenol Clenbuterol Fenoterol	-	↓	stimulant, insomnia	(S28-S30)
Antagonists				
Alpha1				
Nicergoline Prazosin Ifenprodil 5-methyl-urapidil Doxazosin L-765314	↑	↑	increased wake, mixed effects on REM sleep	(S30-33)
Beta				
Bopindolol Carvedilol	-	↑	reduced activity, sedation	(S34, S35)

Figure S9

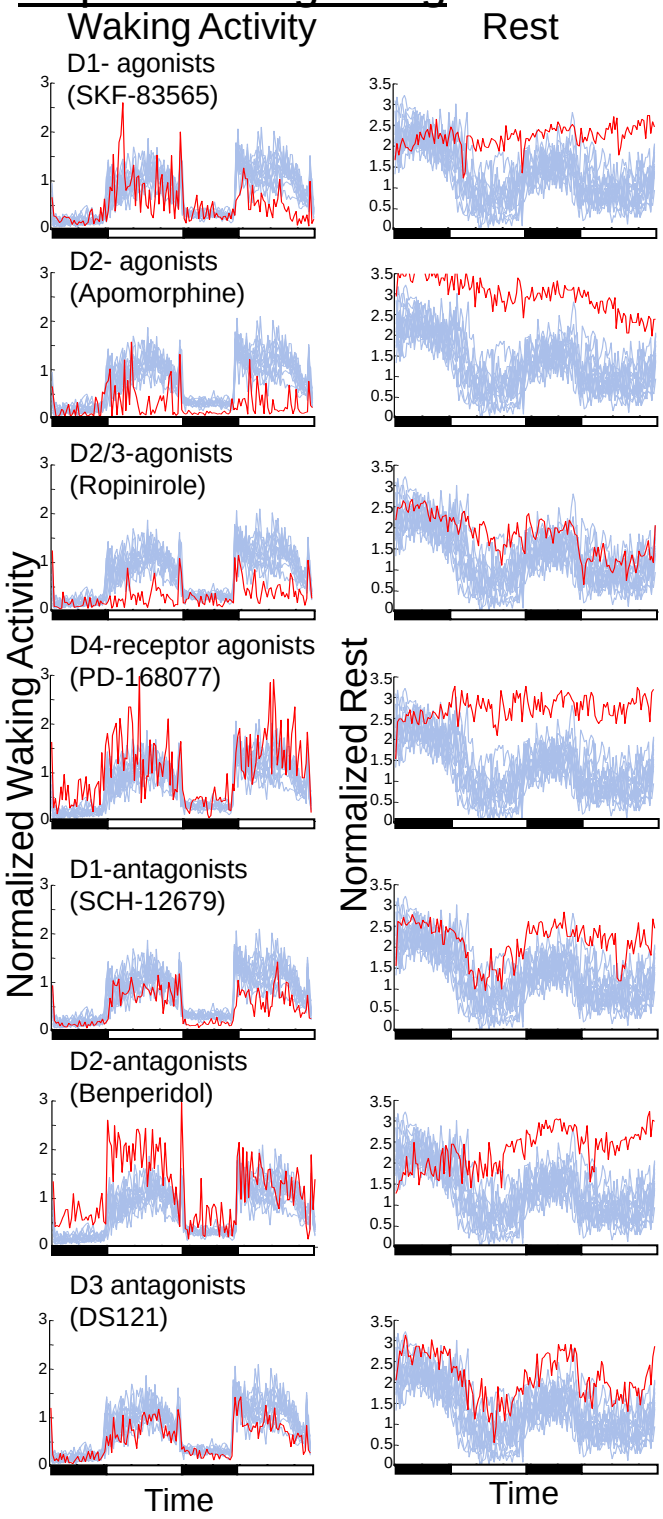
Serotonin Signaling



	Wake	Rest	Effects in mammals	Selected References
Agonists				
5HT-1A BMY-7378 BP-554 Buspirone PAPP RU-24969	- ↑ -	↑	short term increases in waking; long term increases in SWS (humans, rats, cats)	(S36-40)
5HT-1B/D CGS-12066A	↓	-	short term increases in waking followed by general deactivation	(S41,42)
5HT-1D Dihydroergotamine GR 46611	↑	↓/↑	migraine treatment potentiates 5HT-1A induced locomotion in guinea pig	S43
5HT-2/3 N-methylquipazine Quipazine	-/↓	↑	short-term sleep suppression; long term increase in SWS	(S38,S44)
Antagonists				
5HT-1A MM-77 NAN-190 P-MPPF S(-)-UH-301 Spiroxatrine WAY-100635	-/↑	↑	reduced locomotion; initial sleep suppression followed by long term SWS increase	(S36,45-47)
5HT-1D Metergoline	↑	↓/↑	short-term insomnia in cats	S48
5HT-2/3 Cinanserin Ketanserin LY-53857 Norcyclobenzaprine	↑	↑	increased sleep; increased exploration	(S38, S49-52)
Reuptake Inhibitors 6-Nitroquipazine Fluoxetine Fluvoxamine Paroxetine Zimelidine	↓	↑	improves insomnia in patients; REM suppression; biphasic effects on SWS in rats/cats -- up short term, down long term	(S53-55)

Figure S10

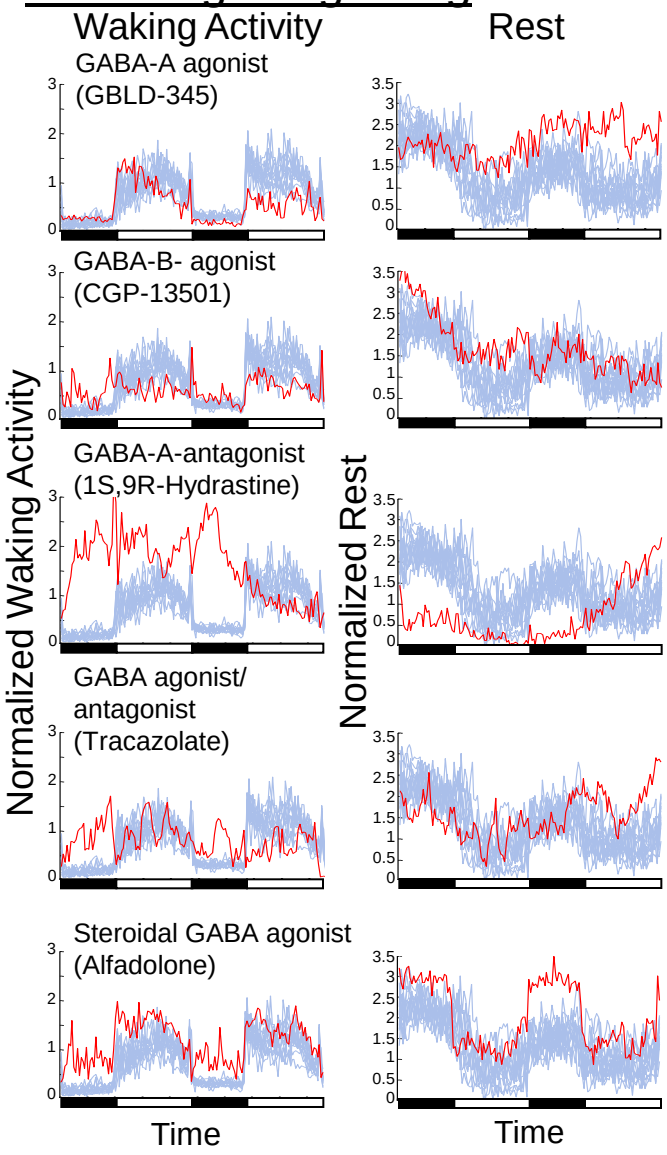
Dopamine Signaling



Agonists	Wake	Rest	Effects in mammals	Selected References
D1-Receptor Chloro-APB CY 208-243 R(+)-6-Bromo-APB N-Allyl-SKF-38393 SKF 83565			increased waking, reduction in sleep (rat and monkeys), and increased grooming	(S56-59)
D2-Receptor Apomorphine Bromocriptine Dipropyl-6,7-ADTN Pergolide (-)-3PPP	↓	↑	biphasic; at low concentrations, reduces waking and locomotor activity, and increases sleep. At high doses, the opposite.	(S60-63)
D2/3-Receptor Quinelorane Quinpirole Ropinirole	↓	- - -/↑	reduced waking; no significant changes in sleep	(S42,S64)
D4-Receptor PD 168077	↑	↑	non-stereotyped shuffling in rats	S65
Antagonists				
D1-Receptor R(-)-SCH-12679 SCH-23390	-	↑	decreased waking, increased sleep	(S56-59, S61,S66)
D2-Receptor Benperidol Droperidol Domperidone L-741626	↑	↑	extra-pyramidal effects (movement disorders); increased sedation	(S57,S61, S66)
D3-Receptor DS121 GR 103691	-/↑	↑	short term increase in waking followed by long-term increases in sleep amount	S65

Figure S11

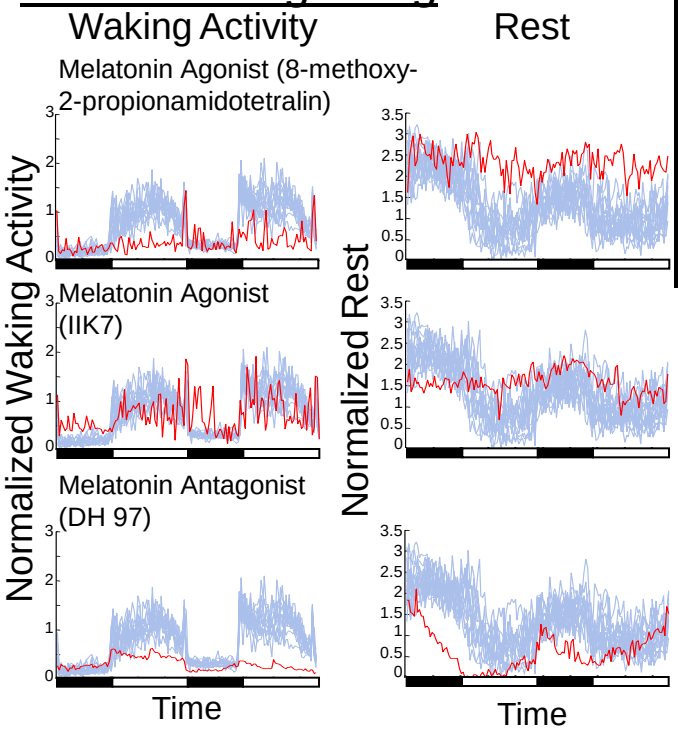
GABAergic Signaling



	Wake	Rest	Effects in mammals	Selected References
Agonists				
GABA-A				
GBLD-345	-	↑	anxiolytic; sedative	S68
Avermectin B1	-			
GABA-B				
CGP-13501			hypnotic	S69
Antagonists				
GABA-A				
1S,9R-Hydrastine Endosulfan Heptachlor Chlordane Heptachlor Aldrin	↑	↓	increased locomtor activity; induction of fighting; pro-convulsant	(S70-72)
Mixed (+/-) Modulator				
Tracazolate	↑	-	anxiolytic; dose dependant increase or decrease in locomotor activity	(S73,74)
Neuroactive Steroid GABA Regulators				
Allopregnanolone Pregnanolone	↑	↑	stimulant; anxiolytic; ataxic; depressant	(S75-77)
Alfadolone		-	hypnotic	

Figure S12

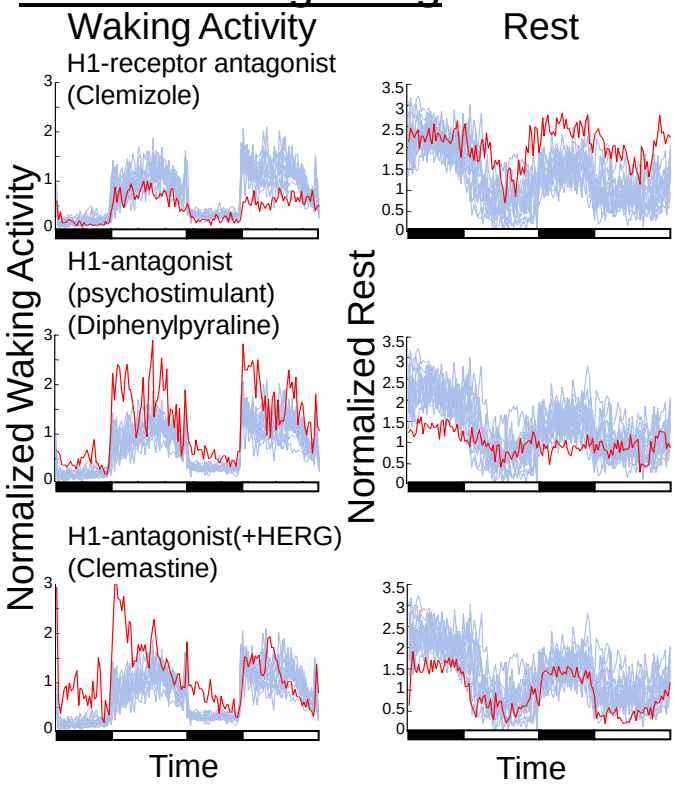
Melatonin Signaling



	Wake	Rest	Effects in mammals	Selected References
Agonists				
8-methoxy-2-propionamidotetralin	↓	↑	increase in sleep propensity in dirunal species; circadian phase shifting	(S78-81)
Melatonin	-	↑		
IIK7	↑	↓/-		
Antagonists				
K 185	↓/-	↓	blocks melatonin effects on behavior	S82
DH 97	↓	↓		

Figure S13

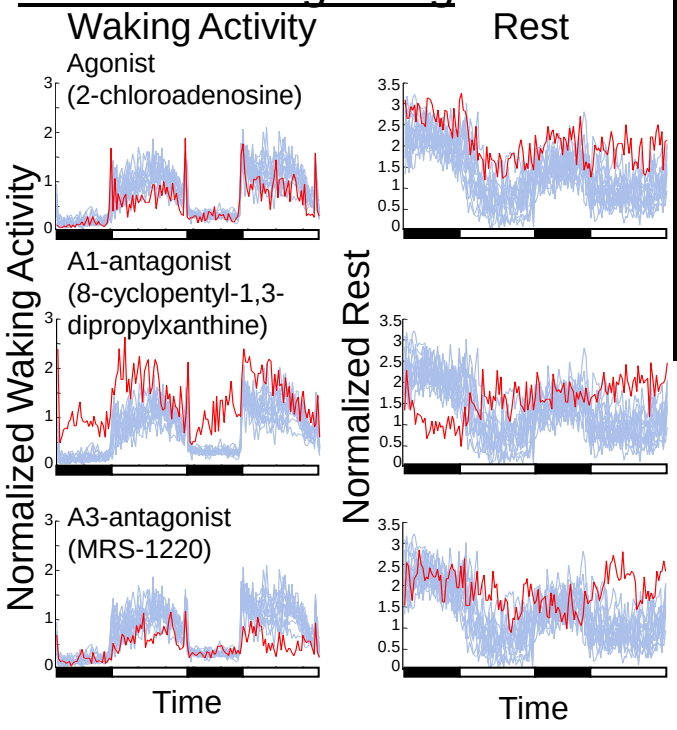
Histamine Signaling



Antagonists	Wake	Rest	Effects in mammals	Selected References
H1-(no HERG block)				
Chloropyramine	-	↑	sedation (less pronounced in loratadine)	(S83-86)
Clemizole	-/↓	↑		
Loratadine	↓	-		
Trimeprazine	↓	↑		
Diphenylpyraline	↑	↓	psychostimulant	S87
H1- (HERG Blockers)				
Astemizole	↑	↑	some sedation in 1st generation anti-histamines; less pronounced in second generation anti-histamines	
Clemastine				
Meclizine				
Oxatomide		↓/-		
Promethazine				
Terfenadine				

Figure S14

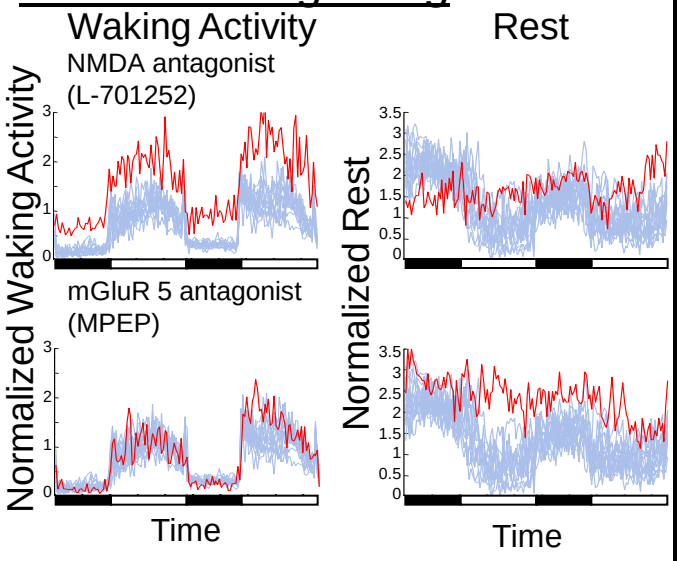
Adenosine Signaling



Agonists	Wake	Rest	Effects in mammals	Selected References
Adenosine 2-chloroadenosine	-	↑	increased sleep; decreased locomotor activity	(S88-92)
Antagonists **A1** 1,3-diethyl-8-phenylxanthine 8-cyclopentyl-1,3-dipropylxanthine	↑	↓	locomotor stimulant	(S88,93)
A3 MRS-1220	-/↓	↑	unknown behavioral effects	

Figure S15

Glutamate Signaling



Antagonists	Wake	Rest	Effects in mammals	Selected References
NMDA DCQX 7-chlorokynurenic acid CNS-1102 IEM 1460 L-689560 L-701252 L-701324 MK-801 SDZ 220-581	↑	-/↓	anxiolytic; hyperlocomotion; anti-convulsant	(S94,95)
mGluR5 MPEP SIB 1757	-/↓	↑	anxiolytic; can block drug induced hyperlocomotion	(S96,97)

Figure S16

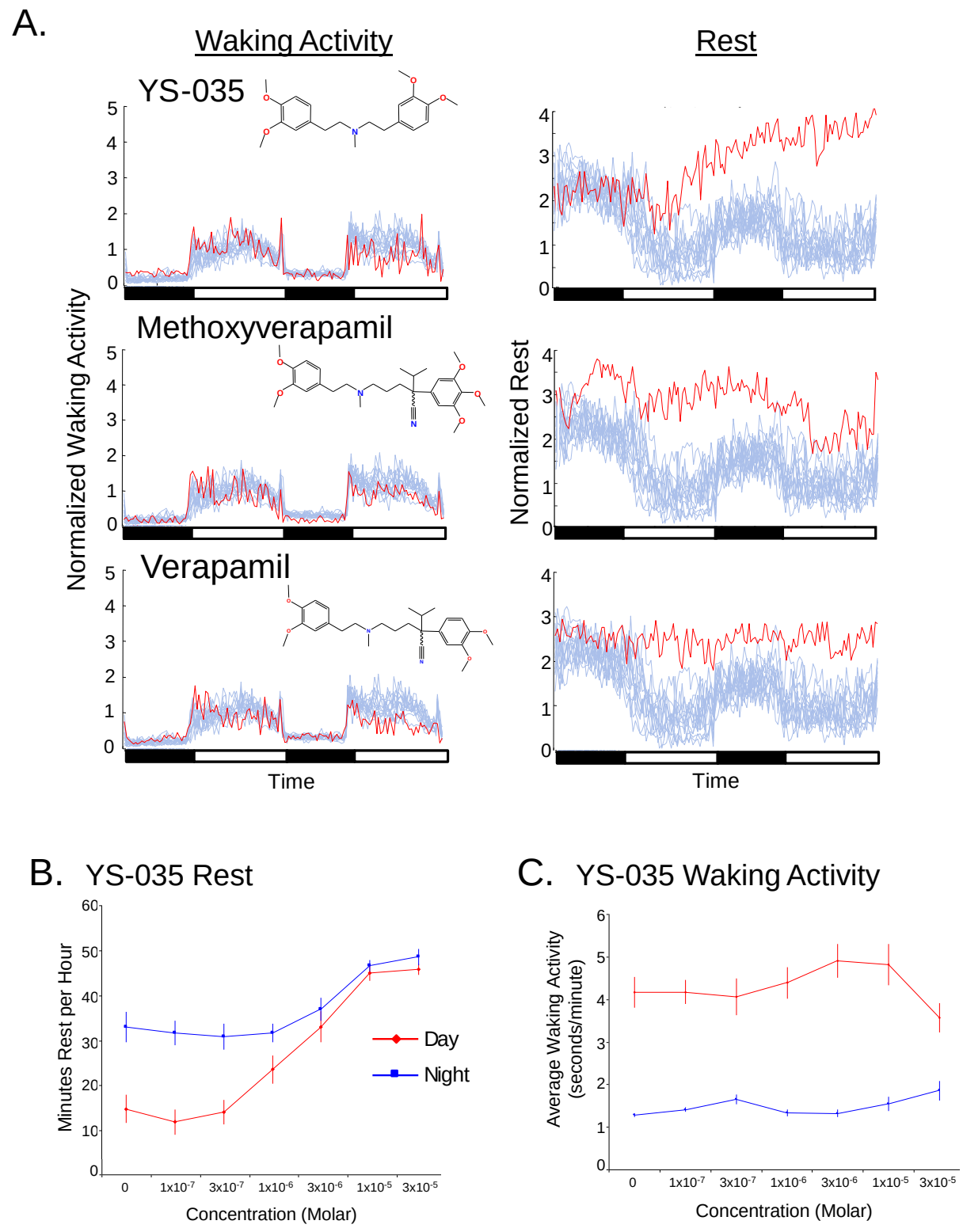

A.
Waking Activity
Rest
YS-035
Methoxyverapamil
Verapamil
Normalized Waking Activity
Normalized Rest
Time
Time
B. YS-035 Rest
Minutes Rest per Hour
Day
Night
Concentration (Molar)
C. YS-035 Waking Activity
Average Waking Activity (seconds/minute)
Concentration (Molar)

Figure S17.

A.

Glucocorticoids
betamethasone
clobetasol
desoxycorticosterone
dexamethasone
flumethasone
flunisolide
hydrocortisone
medrysone
6α-methylprednisolone
ursolic acid

PDE inhibitors
diplosalsalate
fosfosal
papaverine
propentofylline
Ro 20-1724
SKF-94836

Non-Steroidal Anti-Inflammatory drugs (NSAIDs)
bufexamac
diflunisal
fenoprofen
piroxicam

Other Anti-inflammatory agents
aminophenazone
capsazepine
catechin tetramethyl ether
cepharanthine
pentamidine
theaflavin digallate
valproate sodium
5-aminopentanoic acid

Immunomodulator (NFAT signaling)
cyclosporin A

B.

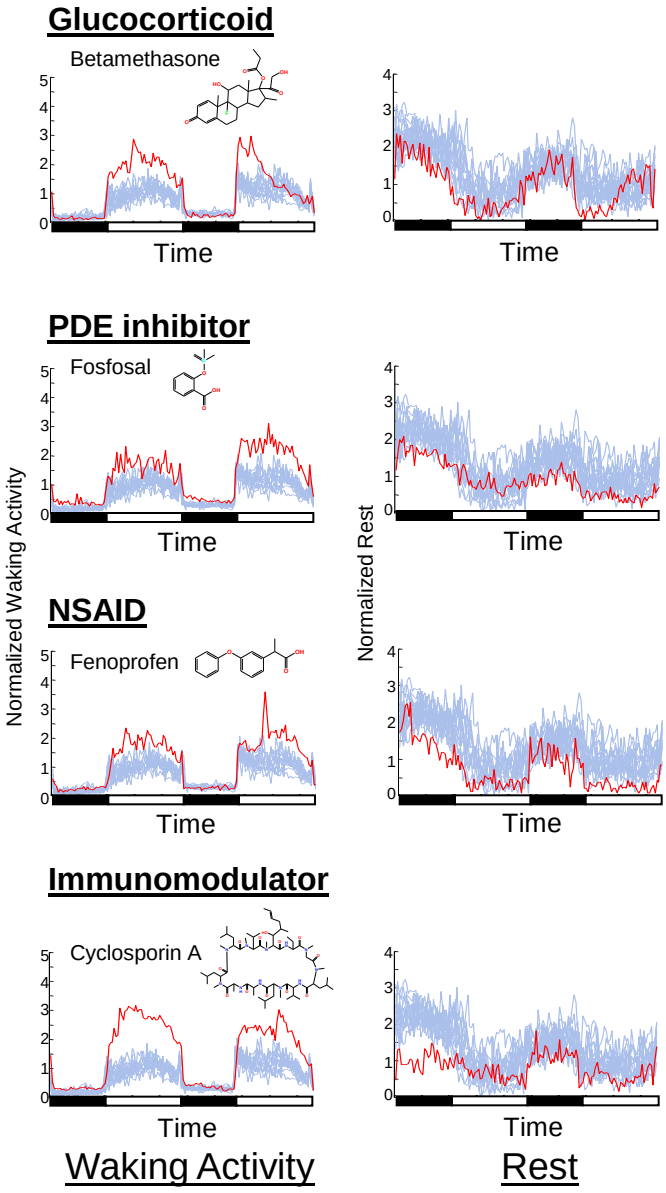
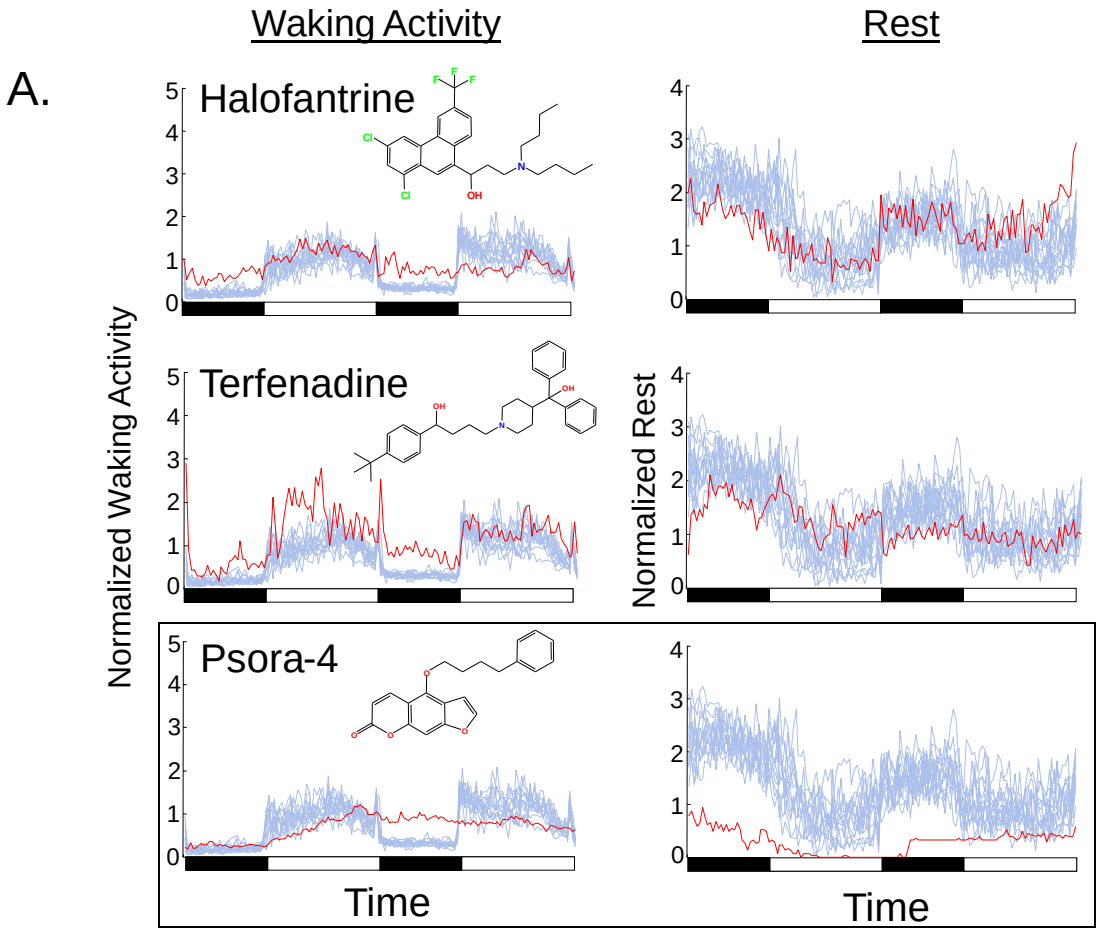


Figure S18


A.
Waking Activity
Rest
Halofantrine
Terfenadine
Psora-4
Normalized Waking Activity
Normalized Rest
Time
Time

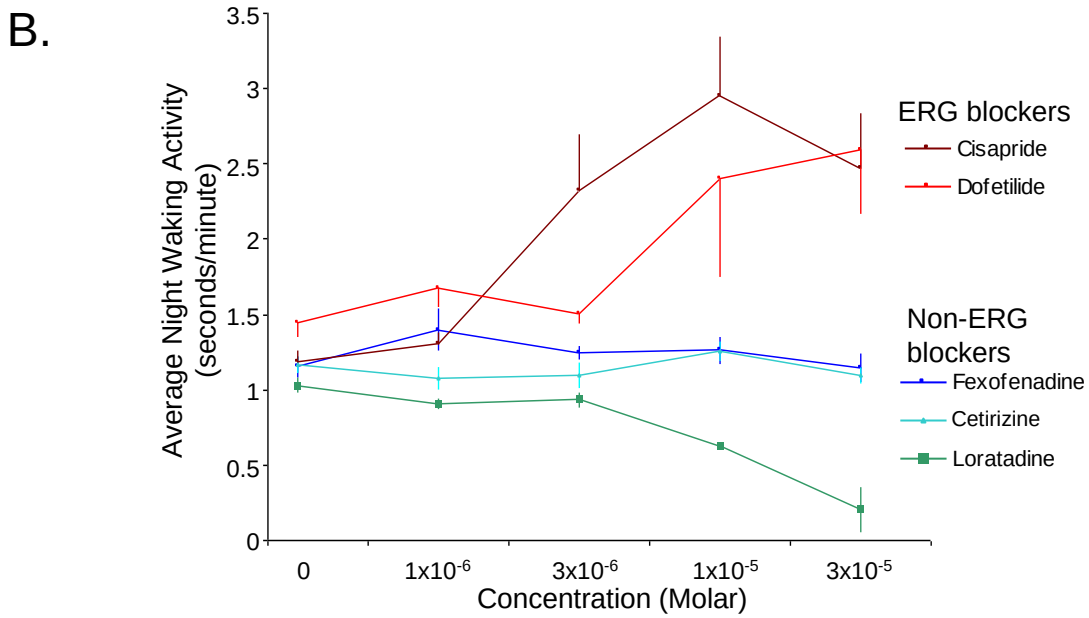

B.
Average Night Waking Activity (seconds/minute)
Concentration (Molar)
0
1x10-6
3x10-6
1x10-5
3x10-5
ERG blockers
Cisapride
Dofetilide
Non-ERG blockers
Fexofenadine
Cetirizine
Loratadine

Table S1

Drug Name	Highest Non-Toxic Dose (30/45 μM max)	Lowest Effective Dose	Correlated Dose Range
Clonidine	45 μM	1.5 μM	1.5-45 μM
Ropinirole	45 μM	0.45 μM	0.45-45 μM
NAN-190	30 μM	0.10 μM	0.10-30 μM
MK-801	45 μM	0.45 μM	0.45-45 μM
Betamethasone	45 μM	0.15 μM	0.15-45 μM
Chloro-APB	45 μM	0.45 μM	0.45-45 μM
Buspirone	45 μM	4.5 μM	4.5-45 μM
Cyclosporin A	45 μM	2 μM	2-45 μM
Ketanserin	15 μM	0.15 μM	0.15-45 μM
Nicergoline	15 μM	1.5 μM	4.5-45 μM
Quipazine	45 μM	1.5 μM	0.15-45 μM
Warfarin	45 μM	4.5 μM	4.5-45 μM
Fluvoxamine	45 μM	1.5 μM	0.45-45 μM
Valproic Acid	45 μM	15 μM	0.45-45 μM
Carvedilol	4.5 μM	0.45 μM	0.45-4.5 μM
2-Chloroadenosine	45 μM	45 μM	0.15-45 μM
Phenelzine	45 μM	45 μM	4.5-15 μM
Trazodone	45 μM	1.5 μM	1.5-15 μM
Clozapine	15 μM	1.5 μM	1.5-15 μM
Amoxapine	30 μM	3.0 μM	1.0-10 μM
Cyclobenzaprine	15 μM	4.5 μM	1.5-15 μM
Fluoxetine	15 μM	1.5 μM	1.5-4.5 μM
Haloperidol	15 μM	1.5 μM	0.15-1.5 μM
Loxapine	15 μM	0.45 μM	0.15-4.5 μM
Papaverine	3.0 μM	1.0 μM	0.30-3.0 μM
Pergolide	45 μM	0.45 μM	4.5-45 μM; 0.15-1.5 μM
Bromocriptine	15 μM	0.15 μM	1.5-15 μM; 0.15-1.5 μM
Dihydroergotamine	45 μM	0.15 μM	4.5-45 μM; 0.15-1.5 μM
Metergoline	15 μM	15 μM	4.5-15 μM; 0.15-1.5 μM

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